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CONTENTS

No. 1. JANUARY

No. 2. FEBRUARY

No. 3. APRIL

No. 4. MAY

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Resumen por el autor, R. W. Hegner.
Universidad Johns Hopkins, Baltimore

Las relaciones entre el número de núcleos, la masa de cromatina, la masa citoplásmica y los caracteres de la concha de cuatro especies del género Arcella.

El presente trabajo contiene observaciones y experimentos sobre Arcella denta, A. polypora, A. vulgaris y A. discoides. Ejemplares de clones cuyo tamaño y número de espinas era conocido se cortaron en dos pedazos. Los pedazos uninucleados de los padres binucleados produjeron descendientes uninucleados que tenían aproximadamente la mitad del tamaño de los padres. Esto indica, por consiguiente, la relación de una cantidad definida de citoplasma con un solo núcleo. Las masas decromatina fueron medidas en ejemplares procedentes de clones de A. dentata que diferían en tamaño y en número de espinas; el autor ha comprobado que la cantidad de cromatina era menor en los ejemplares procedentes de los clones mas pequeños. Es decir, que la cantidad de citoplasma varía directamente con la cantidad de cromatina. A. polypora contiene de tres a trece núcleos. El número de núcleos varía dentro de un clono, y el tamaño enun mismo clono está relacionado muy estrechamente con el número de núcleos. Los ejemplares con el mismo número de núcleos, pero pertenecientes a clones diferentes pueden diferir en tamaño. Las medidas de las masas de cromatina demuestran que los ejemplares del mismo tamaño pertenecientes a clones diferentes tienen aproximadamente la misma cantidad de cromatina, aunque el número de núcleos sea diferente. Esto indica que la cantidad de citoplasma no depende del número de núcleos, sino de la cantidad total de cromatina. El autor discute los datos obtenidos con referencia a la teoría de la relación núcleocitoplásimca, la teoría de la relación cromatocitoplásmica, las teorías de las líneas puras y de la selección, y la hipótesis de los cromidios.

Translation by José F. Nonidez
Carnegie Institution of Washington

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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THE RELATIONS BETWEEN NUCLEAR NUMBER, CHROMATIN MASS, CYTOPLASMIC MASS, AND SHELL CHARACTERISTICS IN FOUR PIECES OF THE GENUS ARCELLA[1]

ROBERT W. HEGNER

School of Hygiene and Public Health, The Johns Hopkins University

FORTY-SEVEN FIGURES

CONTENTS

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[1] The observations and experiments described in this paper were begun in the zoological laboratory of the Johns Hopkins University on December 27, 1917; were continued at the laboratory of the Brooklyn Institute of Arts and Sciences at Cold Spring Harbor, Long Island, from June 17 to August 27, 1918, and were completed at the School of Hygiene and Public Health of the Johns Hopkins University. The writer is indebted to the members of the Zoological Department of the Johns Hopkins University for many courtesies and to Dr. C. B. Davenport for the opportunity of carrying on work at Cold Spring Harbor. He is particularly grateful to Prof. H. S. Jennings for his valuable counsel. Much of the statistical work was done by Dr. Ruth Stocking Lynch, instructor in Protozoology in the School of Hygiene and Public Health.

1. INTRODUCTION

a. The problem

The investigations described in the following pages were undertaken to determine the relations between nuclear number, chromatin mass, cytoplasmic mass, and shell characteristics in certain species of the genus Arcella. As a rule, genetic research in animals is limited to the study of somatic characteristics alone or to the examination of germ cells that have been killed and prepared for microscopic observation. In many cases the germ cells of organisms that have been used in breeding experiments have been studied, but these germ cells have been obtained either from control specimens or from pedigree specimens that have been killed for the purpose. Arcella is peculiarly favorable for investigation because both nuclear and cytoplasmic characteristics can easily be seen drawn and measured, at the same time, in the living animal, and their relations can thus be established under the most favorable circumstances.

b. Advantages of Arcella for nucleocytoplasmic studies

In a previous paper (Hegner, '19), various characteristics that make Arcella a favorable organism for genetic investigations were pointed out. Among these are: 1) the power of multiplying vegetatively and rapidly; 2) the presence of definite measurable characters that are not modified by growth; 3) the semitransparency of the shell which makes possible the examination of the contents, especially in the recently formed offspring; 4) the ability to withstand severe operations, and, 5) the ease of cultivation and examination. To this list should be added the fact that the nucleus is of the vesicular type with the chromatin, when in the resting stages, clumped into a spherical mass which may easily be drawn and measured. It is thus possible to study the relation between chromatin and cytoplasm, which offers a much more accurate means of comparison than when the entire nucleus with the nuclear sap is involved.

c. Methods

The methods of rearing the specimens recorded in this paper are the same as those previously described (Hegner, '19). The operations were for the most part simple. The specimens were first drawn with a camera lucida; they were then cut in pieces with a small, sharp scalpel, and the positions of the cuts were indicated on the sketch. In one set of experiments the nucleus only was dissected out with the aid of a Barber microdissection apparatus. Parts of specimens or those from which nuclei had been removed were cultivated as were the entire animals.

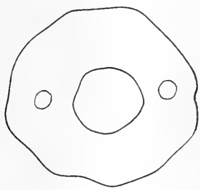
2. EXPERIMENTS ON ARCELLA DENTATA

a. Experiments on binucleate members of family 150

1. Introduction. The progenitor of this family (fig. 1) was taken from a pond on the campus of the Johns Hopkins University at Homewood, Baltimore, on December 27, 1917. Its spines consisted of almost indistinguishable ridges and could not be counted. In diameter it measured 34 units of 4.3μ each. Four immediate offspring were obtained from this specimen, all of which exhibited well-defined spines, showing that the absence of spines in the parent was probably due to some environmental factor. The fourth offspring was represented by an empty shell; the other three possessed 13, 14, and 14 spines, respectively; one was 34 units and the other two were 35 units in diameter (fig. 2).

2. Results of removing part of the shell and part of the cytoplasm. The first experiment was designed to answer the following questions. If part of the shell is removed, is a new part regenerated? What influence on a specimen and its descendants has the removal of part of the cytoplasm and part of the chromidia? The first offspring was operated on as indicated in figure 3. Part of the shell and some of the cytoplasm were removed. No regeneration of the shell occurred. The first offspring produced by this specimen after the operation was smaller than the parent (15–31 in fig. 2), this decrease in size being due probably to the

removal of part of the parental cytoplasm. The first offspring of this specimen, however, was as large as the original parent and the mass relations between nuclei and cytoplasm were thus regained. It may be noted that the protoplasm removed con-



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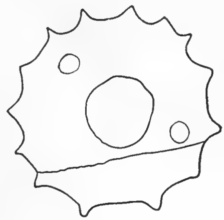

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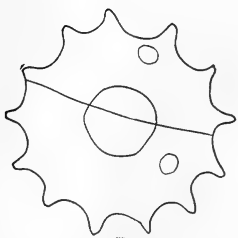

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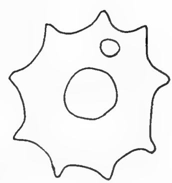

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Fig. 1 Arcella dentata. Outline of the shell of the progenitor of family 150. The inner circle represents the mouth opening. × 207.

Fig. 3 Arcella dentata. Outline of the shell of specimen 150.1. The cross line indicates the portion of the shell and cytoplasm removed. The small circles represent the two nuclei. × 207.

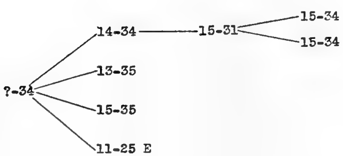
Fig. 5 Arcella dentata. Specimen 150.2. The cross line indicates where it was cut in two. The upper, smaller portion was the progenitor of line 150.2a; the lower, larger portion, of line 150.2b. × 207.

Fig. 6 Arcella dentata. Specimen 150.2bl. The first offspring of the larger portion of specimen 150.2 shown in figure 5. × 207.

tained a portion of the chromidial net which is so conspicuous in Arcella (fig. 4), but there is no evidence that its removal affected the characteristics of the organism.

3. The reproduction of uninucleate pieces. The next problem was to determine the effects on the organism of removing one

nucleus and half of the cytoplasm. The second offspring was cut into two slightly unequal parts, each containing one nucleus (fig. 5). The smaller part was labeled 150.2a and the larger part 150.2b. Both of these parts survived and reproduced. Their immediate offspring were slightly irregular in shape (fig. 6) but exhibited spines that could easily be counted. The effects of injuries to the parental shell upon the shape of the offspring are clearly indicated in figures 7 and 8. The immediate



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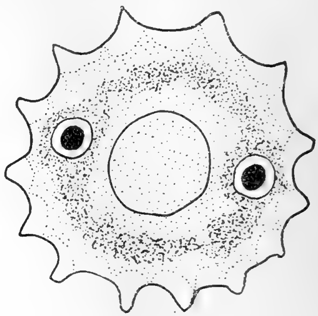

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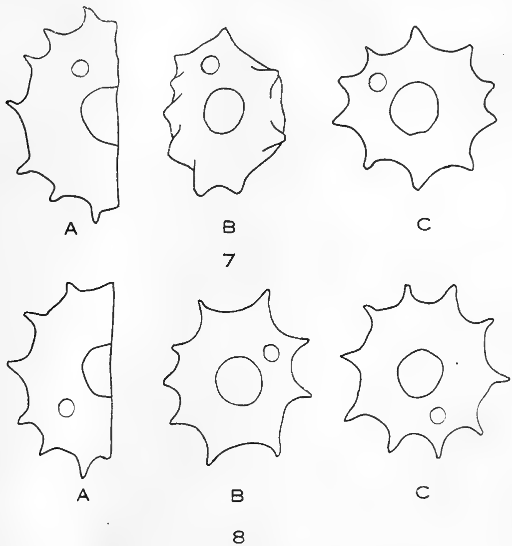
Fig. 2 Arcella dentata. Pedigree of family 150 showing the number of spines and diameter of the shell of the progenitor of the family and of a few of the progeny. Each vertical series of numbers represents a generation. The number preceding the dash is the number of spines and the number succeeding the dash is the diameter of the shell in units of 4.3μ each.

Fig. 4 Arcella dentata. Nuclei, cytoplasm, cytoplasmic attachments to the inside of the shell, and the chromidial net are shown in this figure as they appear in a stained specimen. × 310.

progeny (B) of the half specimen (A) is irregular in shape, but the normal shape (C) is usually regained in the next generation. The condition of the shell of the parent evidently has only a very slight influence upon the shape of the shell of the offspring.

Besides being slightly irregular in shape, these offspring were much smaller than the original, entire parent, and possessed only one nucleus each. Furthermore, the offspring of the smaller part (150.2a) were smaller than those of the larger (150.2b) as is shown in the pedigree in figure 9. This result is similar to that

described in the first experiment, in so far as it indicates that the size of the immediate progeny is, at least in part, dependent upon the amount of cytoplasm in the parent. By the fourth generation (table 1) the specimens in line 150.2a had reached a mean spine number approximately equal to that of line 150.2b and were rapidly approaching the latter in diameter. This sug-



Figs. 7 and 8 Arcella dentata. Specimens of family 150. *A* is one-half of a binucleate specimen; *B*, its first offspring, and *C*, the first offspring of the succeeding generation. The normal shape is usually regained in the second generation. × 207.

gests that the nuclei and cytoplasm in the two lines were qualitatively alike and that their interaction was such as to lead gradually to the production of specimens in which an equilibrium between the nuclear and cytoplasm masses was regained, thus resulting in specimens similar as regards spine number and diameter.

4. Empty shell formation and nuclear doubling. At first it appeared that a smaller, uninucleate line of Arcellas had been established by the cutting experiments, but soon a most interesting phenomenon occurred at the time of division in the case of certain of these uninucleate specimens; this was the forma-

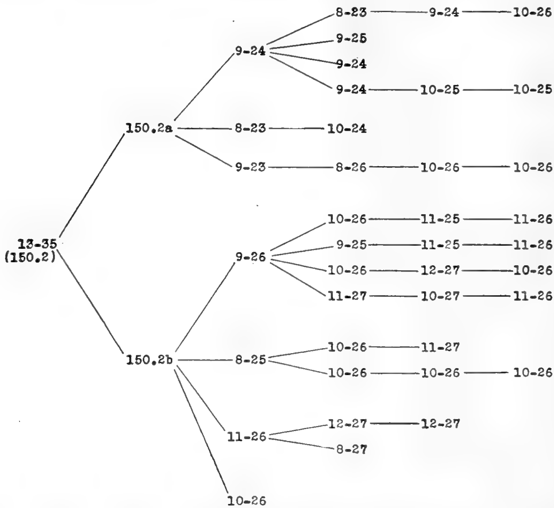


Fig. 9 Arcella dentata. Part of the pedigree of the two lines, 150.2a and 150.2b resulting from the bisection of specimen 150.2.

TABLE 1

Arcella dentata. Table showing differences in mean spine number and in mean diameter for the first four generations of lines 150.2a and 150.2b, exclusive of empty shells and the progeny of binucleate specimens. The unit of measurement is 4.3 μ

LINE	GENERATION 1		GENERATION 2		GENERATION 3		GENERATION 4	
	Mean spine number.	Mean diameter	Mean spine number	Mean diameter	Mean spine number	Mean diameter	Mean spine number	Mean diameter
150.2a	8.67	23.33	9.12	24.37	9.86	24.86	10.80	25.00
150.2b	9.50	25.75	9.91	26.18	10.71	26.43	10.33	26.40

tion of empty shells and the regaining of the binucleate condition. Empty shells were formed at irregular intervals, and in every case the parents, on examination, were found, after empty shells were thrown off, to possess two nuclei instead of a single nucleus. The next offspring produced by these binucleate parents also possessed two nuclei and were always larger and had more spines than their parents or the empty shells (fig. 10). The offspring of these binucleate progeny were

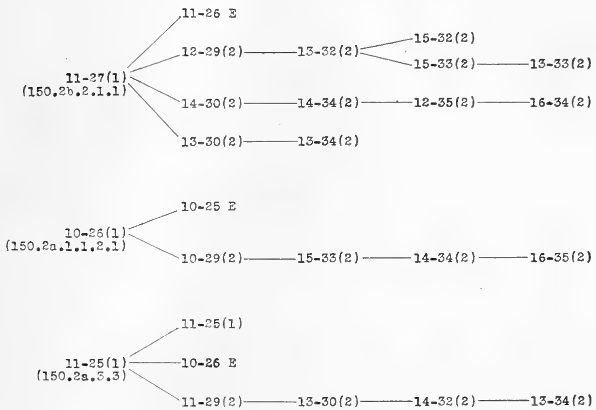


Fig. 10 Arcella dentata. Parts of the pedigrees of specimens belonging to family 150, showing the relative size of uninucleates, empty shells, and binucleates, and the gradual increase in size of the binucleates until the 'normal' dimensions are attained in the third or fourth generations. The empty shells are indicated by the letter E and the number of nuclei by the numbers in parentheses.

likewise binucleate and were again larger and had more spines than their parents. Thus the members of each generation after empty shell formation and nuclear doubling exceeded those of the preceding generation in spine number and in diameter until the specimens attained an average for these characters equal to that of the line before the operation was performed. The attainment of this condition required, as a rule, three or four generations (fig. 11). Tables 2 and 3 give the data for

thirteen cases in both lines, 150.2a and 150.2b. It seems evident that the specimens possessed the potentialities of the original line as soon as they regained the binucleate condition, but that their immediate progeny were probably limited in their

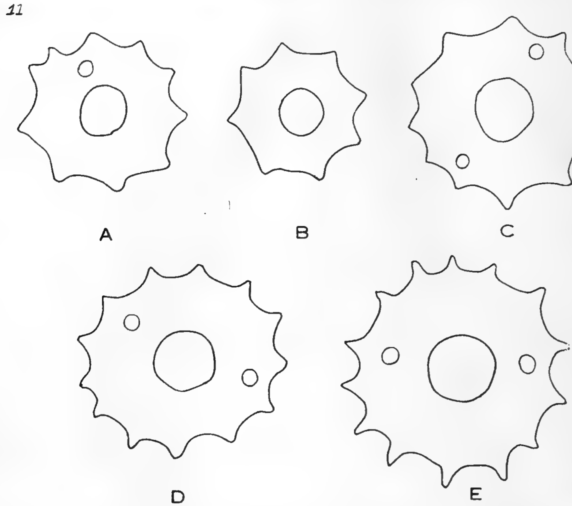


Fig. 11 Arcella dentata. Family 150. Outlines showing the comparative sizes of specimens during the process of nuclear doubling, and the gradual increase in spine number and dimensions during the next three generations until the normal characteristics of the family are regained. *A* = uninucleate specimen no. 150.2a.1.1.3.1.1.1.1. with 10 spines and a diameter of 26 units of 4.3 μ each. *B* = an empty shell (150.2a.1.1.3.1.1.1.1.2) thrown by *A*, with 8 spines and a diameter of 22 units. *C* = the first offspring (150.2a.1.1.3.1.1.1.1.3) produced by *A* after the empty shell was formed, with 11 spines and a diameter of 31 units. *D* = the first offspring (150.2a.1.1.3.1.1.1.1.3.1) of *C*, with 13 spines and a diameter of 33 units. *E* = the first offspring (150.2a.1.1.3.1.1.1.1.3.1.1.) of *D*, with 14 spines and a diameter of 35 units. $\times$ 207.

increase by the amount of cytoplasm within the parent, and thus the full size could not be attained at one step.

There did not seem to be any regularity in the occurrence of nuclear doubling, but the binucleate condition was finally

TABLE 2

Arcella dentata. Table presenting the data for thirteen cases of empty shell formation accompanied by nuclear doubling in lines 150.2a and 150.2b. The data include the diameters and spine numbers of the uninucleate parents, of the empty shells they produced, of the first offspring formed after throwing the empty shells, and of the first offspring of the three succeeding generations. The unit of measurement is 4.3 μ

LINE	PARENT		EMPTY SHELL		FIRST BINUCLEATE		GENERATION 1		GENERATION 2		GENERATION 3	
	Diameter	Spine number	Diameter	Spine number	Diameter	Spine number	Diameter	Spine number	Diameter	Spine number	Diameter	Spine number
150.2b	26	10	26	9	28	12	31	13	31	14	31	13
	26	10	26	11	31	13	35	17	35	15	36	15
	27	10	24	8	31	14	31	13	33	15	36	14
	26	10	25	11	30	13	31	13	34	14	34	15
	26	9	27	10	31	13	31	17	30	14		
	27	11	26	11	29	12	32	13	33	15	33	13
	27	12	27	10	29	14	31	14	34	16	35	16
	27	8	25	10	31	11	34	15	35	16	37	17
150.2a	25	10	27	11	28	10						
	26	10	25	10	29	10	33	15	34	14	35	16
	26	10	22	8	31	11	33	13	35	14	35	14
	24	9	25	9	27	12	32	13	32	14	32	13
	25	11	26	10	29	11	30	13	32	14	34	13

TABLE 3

Arcella dentata. Table showing the mean diameter and mean spine number of thirteen parents, at the time of nuclear doubling in lines 150.2a and 150.2b, and also of the empty shells and of the immediate offspring for four succeeding generations. The unit of measurement is 4.3 μ

	MEAN DIAMETER	MEAN SPINE NUMBER
Parent	26.00	10.00
Empty shell	25.46	9.85
Generation 1	29.54	12.00
Generation 2	32.00	14.08
Generation 3	33.17	14.58
Generation 4	34.36	14.45

regained in every case, although in one branch of line 150.2b thirty-three succeeding generations of uninucleates were produced before an empty shell was formed and nuclear doubling occurred, and in other branches doubling did not take place until after thirty-two, thirty-one and twenty-three uninucleate generations were passed through. Sometimes the first offspring produced by a uninucleate would be empty and the second offspring binucleate, but in other cases 6, 7, 9, 11, and 12 uninucleate progeny were formed by single specimens before nuclear doubling occurred. During the time these lines were being reared three binucleate specimens threw off empty shells. What caused them to do this is unknown, and the process was not accompanied by any nuclear changes within the parents nor followed by any marked modification of the later offspring.

Why the organisms do not remain in a uninucleate condition indefinitely, why an empty shell is formed at the time of nuclear doubling, and how this doubling actually takes place, are questions still to be answered. However, it seems probable that when the factor or factors that initiate this phenomenon become operative the uninucleate specimen undergoes nuclear division and forms a shell, but the shell is cast off empty and the parent retains the two nuclei. From a genetic standpoint it is of particular importance to emphasize the fact that it requires three or four generations before the full diameter and spine number of the line are regained after nuclear doubling, since here a very great single change in the internal condition of the organism is revealed by the body as a series of small changes, and hence the small and gradual heritable changes that have been noted by various investigators who have selected body characters in their breeding experiments, may really have been due to large changes in the germ plasm that were only slowly exhibited by the soma.

5. Correlations. Are diameter and spine number correlated in binucleate specimens and in uninucleate specimens? The correlation between the diameter and spine number in both uninucleate specimens and binucleate specimens within lines 150.2a and 150.2b is marked as shown in tables 4 and 5. The

TABLE 4

Arcella dentata. Correlation table for spine number and diameter of shell of the uninucleate specimens in lines 150.2a and 150.2b. The unit of measurement is 4.3 μ. Coefficient of correlation, 0.402 ± 0.039

DIAMETER OF SHELL	NUMBER OF SPINES						
	8	9	10	11	12	13	
23	2	1				1	4
24		6	1	1			8
25	1	4	8	11	1		25
26	2	5	35	16	4		62
27	1	5	26	37	8	1	78
28			8	10	7	1	26
29			1	1	3	1	6
Total.....	6	21	79	76	23	4	209

TABLE 5

Arcella dentata. Correlation table for spine number and diameter of shell of the binucleate specimens in lines 150.2a and 150.2b. The unit of measurement is 4.3 μ. Coefficient of correlation, 0.640 ± 0.027

DIAMETER OF SHELL	NUMBER OF SPINES									
	10	11	12	13	14	15	16	17	18	
27			1							1
28	1		2	1						4
29	2	2	2	2	1					9
30		1	1	4	3					9
31		2	1	7	10	1		1		22
32			5	6	9	2	1			23
33			2	9	4	14	5			34
34			1	11	13	29	9	2		65
35			1		7	16	11	2		37
36					2	5	2	2		11
37						1		1		2
38						1			1	2
Totals.....	3	5	16	40	49	69	28	8	1	219

mean diameter and mean spine number of these specimens are as follows:

	NUMBER OF SPECIMENS	MEAN SPINE NUMBER	MEAN DIAMETER
Binucleates	219	14.25	33.16
Uninucleates	209	10.48	26.45

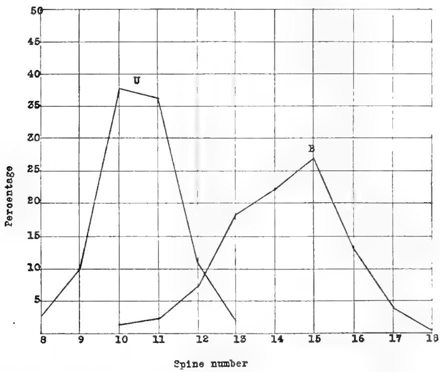


Fig. 12 Arcella dentata. Curves for the variations in number of spines in the uninucleates (*U*) and binucleates (*B*) of lines 150.2a and 150.2b, plotted from the data of tables 4 and 5. The ordinates are percentages and the abscissae numbers of spines.

The variations in spine numbers and diameter between uninucleates and binucleates are indicated graphically in figures 12 and 13.

The coefficient of correlation between spine number and diameter is for the uninucleates 0.402 ± 0.039, and for the binucleates, 0.640 ± 0.027.

6. Changes from the binucleate to the uninucleate condition. Several interesting changes from the binucleate to the uninucleate condition and back again occurred in line 150.2b. The pedigree of one of these is shown in figure 14. In this case a binucleate specimen with 12 spines and a diameter of

32 units gave rise to a uninucleate with 14 spines and a diameter of 35 units. This specimen produced a smaller uninucleate with 11 spines and a diameter of 28 units, and this then passed through empty shell formation and nuclear doubling, and finally produced a binucleate with normal characters. The change from the binucleate condition to the uninucleate condition was probably due to the failure, for some unknown reason, of

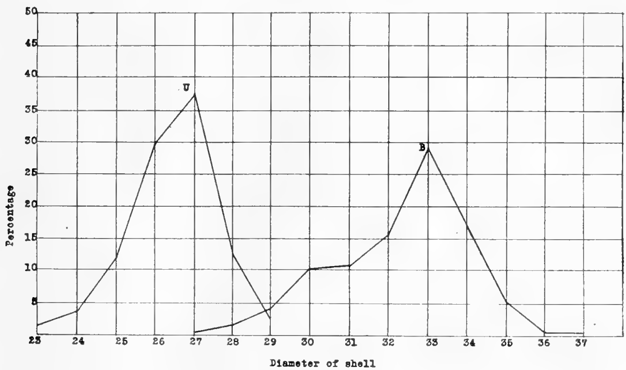
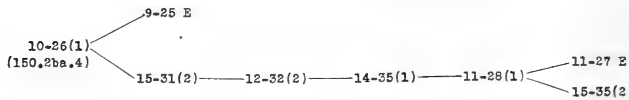


Fig. 13 Arcella dentata. Curves for the variations in diameter in the uninucleates (U) and binucleates (B) of lines 150.2a and 150.2b plotted from the data of tables 4 and 5. The ordinates are percentages and the abscissae numbers of spines.

the nuclei of the parent to divide, hence the offspring obtained one and the parent retained the other. The uninucleate offspring was in the normal binucleate condition as regards spine number and diameter because of the large mass of cytoplasm in the parent. No doubling of the mass of cytoplasm probably took place in the large uninucleate offspring because the quantity that could react properly with a single nucleus was already present. The offspring of this large uninucleate was therefore smaller and nearer the average condition of the uninucleates of the family. However, it contained a mass of cytoplasm great

enough to initiate the process of empty shell formation and nuclear doubling, which occurred immediately.

The other case whose pedigree is shown in figure 15 is somewhat similar. A binucleate specimen gave rise to a smaller uninucleate offspring and itself became uninucleate during the process. It then produced another small uninucleate; and followed this by empty shell formation and nuclear doubling, after which its offspring were all normal binucleates. It will



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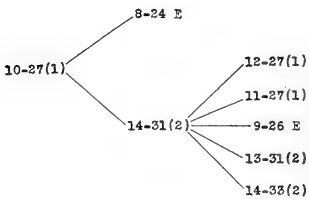

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Fig. 14 Arcella dentata. Part of pedigree of line 150.2b, showing spine number and diameter of specimens during reversion from the binucleate to the uninucleate condition and back again. *E* = empty shell. The numbers enclosed by parentheses indicate the number of nuclei present.

Fig. 15 Arcella dentata. Part of pedigree of line 150.2b, showing spine number and diameter of specimens during reversion from the binucleate to the uninucleate condition and back again. *E* = empty shell. The numbers enclosed by parentheses indicate the number of nuclei present.

be noted that the first uninucleate was small, differing in this respect from that described above. This may be explained by the failure of the binucleate parent to produce enough cytoplasm to form two binucleates. Outlines of the specimens described in this case of reversion are shown in figure 16. Nuclear division may in these organisms be initiated when a certain cytoplasmic mass has been reached, and the lack of cytoplasm may account for the failure of the nuclei to divide in this case.

7. Reproduction of a uninucleate piece formed without an operation. Line 159. Arcellas in their natural environment are probably subjected to injuries very much like those resulting

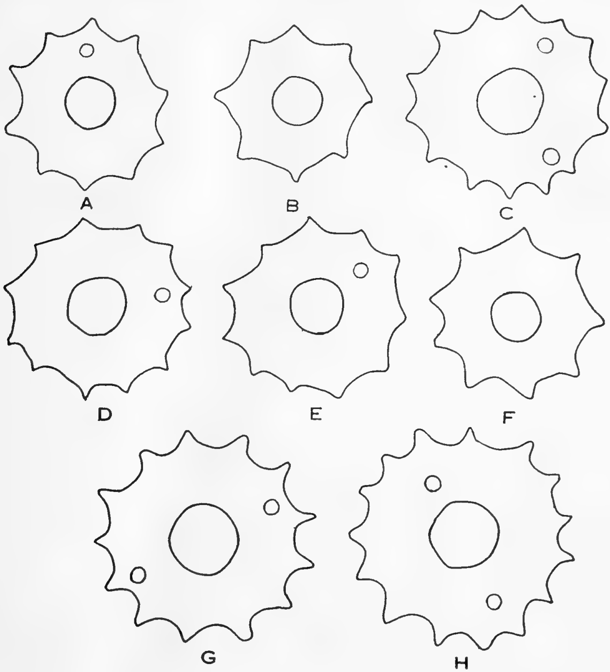


Fig. 16 Arcella dentata. Outlines of the specimens in the pedigree shown in figure 15. The drawings can be identified by comparing the following numbers and letters with those used in the pedigree. *A* = 10–27 (1). *B* = 8–24 E. *C* = 14–31 (2). *D* = 12–27 (1). *E* = 11–27 (1). *F* = 9–26 E. *G* = 13–31 (2). *H* = 14–33 (2). × 207.

from the operations described above, and it is also conceivable that mechanical factors might interfere with normal fission in such a way as to cause various abnormalities. It was interesting, therefore, to discover on January 18, 1918, that one of

the binucleate members of family 150 (fig. 17, *A*) had produced a specimen with only one-half of a shell and with only one nucleus (fig. 17, *B*). The later offspring of this parent were entirely normal (fig. 17, *C*). Fifty descendants consisting of thirty-five uninucleates, one empty shell, and fourteen binucleates were reared from this uninucleate half-specimen, and these were in every way similar to those reared from similar pieces obtained by cutting operations. One uninucleate from this line was bisected, and twenty-four descendants were obtained from the nucleated portion. As in other similar experiments to be described later, uninucleate offspring were at first

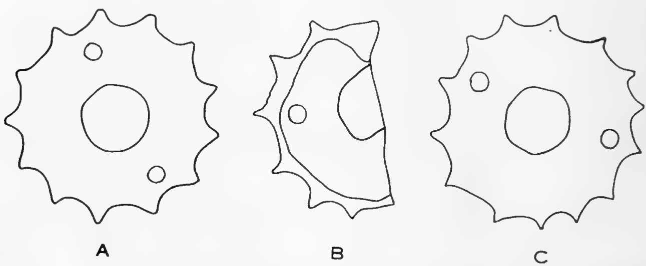


Fig. 17 Arcella dentata. Outline drawings showing the binucleate parent (*A*) of the uninucleate half-specimen (*B*), and one of the binucleates (*C*) from the line derived from this half-specimen. × 207.

produced, but later, empty shells were formed and binucleates appeared which finally reached a diameter and spine number characteristic of the family 150.

8. Microdissection experiments. What effect does the removal of one nucleus and no cytoplasm have upon the specimen and its progeny? In the experiments already described cytoplasmic substances as well as nuclei were removed. It was found possible, however, to remove the nuclei alone by means of a Barber microdissection apparatus.[2] If a very small portion of the edge of the shell is broken away near where a nucleus is located

[2] The writer is indebted for the use of this instrument to Dr. Warren Lewis, of the Johns Hopkins Medical School.

(fig. 18), the latter may be squeezed out very easily and in most cases is free from any accompanying cytoplasm. Small lines were reared from thirteen full-sized binucleate specimens that were successfully operated upon. These specimens were taken from line 150.2a or 150.2b or from lines derived from them. In eight of the thirteen specimens the first offspring, after the

Fig. 18 Arcella dentata. Outline of a specimen showing where the shell was broken away by means of the microdissection apparatus. The nucleus nearby was squeezed out through the opening in the shell. × 207.

operation, consisted of an empty shell and the binucleate condition was immediately regained. Part of the data for these eight cases is as follows:

NUMBER OF EXPERIMENT	PARENT		FIRST OFFSPRING EMPTY		SECOND OFFSPRING BINUCLEATE	
	Diameter	Spine number	Diameter	Spine number	Diameter	Spine number
4	35	16	30	13	33	13
5	35	14	31	12	33	14
18	34	14	28	11	34	13
19	35	15	28	12	33	14
20	33	14	27	11	30	12
21	34	15	27	11	33	12
22	34	14	26	10	35	12
23	35	16	24	11	32	14

When these data are compared with those of the lines already described (fig. 10 and tables 2 and 3), it will be noted that almost all of the empty shells were larger and possessed more spines, and the first offspring after the binucleate condition was regained

were also larger and possessed more spines. This may be brought out more clearly by comparing the means for lines 150.2a and 150.2b with those for the thirteen experimental lines in this series as follows:

	EMPTY SHELLS		UNINUCLEATES		BINUCLEATES	
	Mean diameter	Mean spine number	Mean diameter	Mean spine number	Mean diameter	Mean spine number
Lines 150.2a and 150.2b	25.46	9.85	26.45	10.48	33.16	14.25
Microdissection series	27.39	11.48	27.60	11.50	32.58	13.51

In this table the greater mean diameter and mean spine number of the empty shells and uninucleates in the microdissection series are quite striking. The binucleate specimens belonged mostly to the first offspring after doubling, which accounts for their smaller diameter and fewer spines. From these data the following conclusions are reached. The large amount of cytoplasm induces the immediate or very early formation of an empty shell and the accompanying nuclear doubling. It likewise is responsible for the greater diameter and spine number of both the empty shells and of the uninucleate specimens. These results are probably brought about because of the inability of the single nucleus to control the metabolism of such a large mass of cytoplasm.

The full diameter and spine number characteristic of the binucleate specimens belonging to family 150 were reached by the second or third generations in these experimental lines. The rapidity with which this normal condition was attained was no doubt due also to the great initial amount of cytoplasm present.

The pedigree of experiment 3 (fig. 19) is given as a sample of what occurred in the remaining five of the thirteen experimental lines, and the pedigree of experiment 4 is presented (fig. 20), because several transitions from the binucleate condition to the uninucleate condition appeared in this line. As in similar cases cited previously, this condition was probably brought about by the failure of the nuclei to divide at the time of fission, and hence the parent and daughter each received a single nucleus.

The principal differences between specimens that have been deprived of one nucleus and those from which one nucleus plus half of the cytoplasm has been removed, seem to be due to the larger amount of cytoplasm in the former. The nucleus and cytoplasm remaining in the latter are able to build up within three or four generations a new nucleus and an equal amount of cytoplasm, thus regaining the original condition, whereas in specimens from which one nucleus alone has been removed, the offspring are larger because of the large amount of cytoplasm in the operated parent and nuclear doubling is hastened, probably being initiated by the inequality between the single nucleus and the cytoplasmic mass.

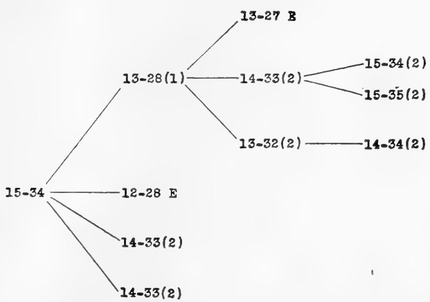


Fig. 19 Arcella dentata. Pedigree of part of the specimens reared in one of the microdissection experiments (exp. 3).

Another series of microdissection experiments consisted in the removal of parts of the nucleus of uninucleates. Operations of this kind were successful, but the patient died in every case. These results are striking when contrasted with those obtained by the removal of cytoplasm, described in the following paragraphs.

b. Experiments on uninucleate specimens of family 150

These experiments were designed to test further the persistence of the relations between nuclei, cytoplasm, and shell characteristics in family 150. Operations were performed

successfully on 6 uninucleate specimens, the operations consisting in the bisection of the specimens so that one part contained the nucleus. In every case the piece without a nucleus died, but that with the nucleus lived and gave rise to uninucleate offspring.

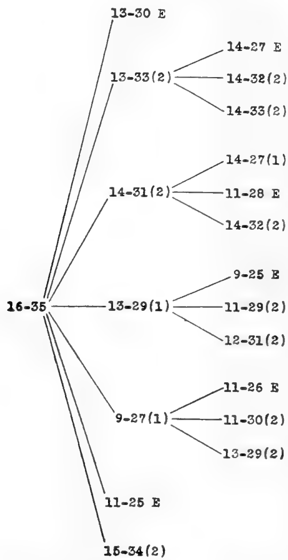


Fig. 20 Arcella dentata. Pedigree of part of the specimens reared in one of the microdissection experiments (exp. 4). Note the reversion from the binucleate to the uninucleate condition in the case of the fourth offspring of the original progenitor.

Experiment 1, line 158a. *Progenitor:* Fourth offspring (uninucleate) of specimen 150.2b; spines, 10; diameter, 26 units (fig. 21, A).

Descendants: Uninucleates, 38; empty shells, 3; binucleates, 11.

Reproduction: As rapid as before the operation.

Immediate progeny: Smaller than original progenitor and with fewer spines; 7 in number, with mean spine number of 9 and mean diameter of 23.14.

Uninucleate descendants: These increased gradually in diameter and in spine number for three or four generations until the normal condition

for the uninucleates of the parent line was regained. This may be illustrated as follows:

NUMBER OF GENERATION	NUMBER OF SPECIMENS	MEAN SPINE NUMBER	MEAN DIAMETER
1	7	9.00	23.14
2	9	9.67	24.77
3	8	9.38	25.50
4	11	10.91	26.91

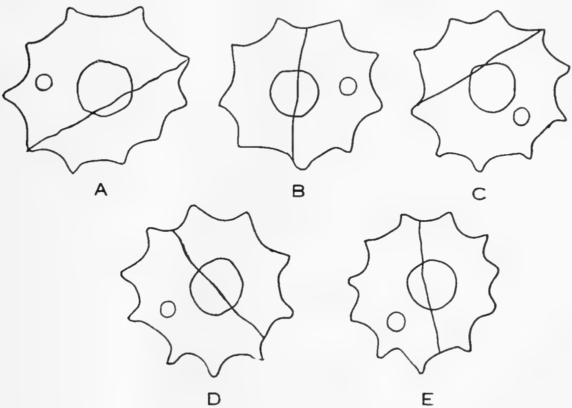


Fig. 21 Arcella dentata. Outlines of a series of uninucleates that were bisected in successive generations. *A* is the fourth offspring of specimen 150.2b. *B* is a specimen from the third generation derived from the nucleated part of specimen *A*. *C* is a specimen derived from the nucleated part of specimen *B*. *D* is a specimen derived from the nucleated part of specimen *C*. *E* is a specimen derived from the nucleated part of specimen *D*. × 207.

Empty shell formation and nuclear doubling: Empty shells were produced by three members of the second generation, the first after having produced three uninucleate progeny, the second after having produced one uninucleate offspring, and the third immediately. In each case nuclear doubling accompanied empty shell formation.

Binucleate descendants: These were, from the first, larger than the uninucleates and gradually increased in diameter and spine number until, within three or four generations, they regained the condition characteristic of binucleates of the parent line, 150.

Experiment 2, line 158alb. *Progenitor:* First offspring (uninucleate) of the nucleated piece of uninucleate specimen 158a; spines, 9; diameter, 24 units.

Descendants: Sixty-four uninucleates were recorded; these belonged to sixteen generations and were produced during a period of nearly two months (January 15 to March 4, 1918). No empty shells were formed and no binucleate specimens occurred during this period, but no doubt they would have appeared if the line had been kept longer. The following table gives the mean diameters and spine numbers of the sixty-four offspring arranged according to generations.

NUMBER OF GENERATION	NUMBER OF SPECIMENS	MEAN SPINE NUMBER	MEAN DIAMETER
1	3	7.00	18.33
2	19	8.26	21.84
3	7	10.00	24.86
4–5	22	10.05	26.09
6–16	13	10.23	26.38

These data show in a very striking manner the gradual increase in diameter and spine number during the first three or four generations, and the attainment in later generations of a mean diameter and a mean spine number characteristic of the uninucleates of family 150. Since the nucleus is the same after the operation as before, we must attribute the small size of the progeny of the first generation to limitations due to the small amount of cytoplasm present. Apparently the nucleus is capable of reacting with a certain amount of cytoplasm, and when, after three or four generations the maximum quantity of cytoplasm has been formed, equilibrium is attained and no further increase takes place. The quantity of cytoplasm may in turn be dependent upon the capacity of the shell.

Experiment 3, line 161a. *Progenitor:* Uninucleate specimen from third generation of line 158a, spines, 9; diameter, 25 units (fig. 21, *B*).

Descendants:

	NUMBER OF SPECIMENS	MEAN SPINE NUMBER	MEAN DIAMETER
Uninucleates	8	10.13	25.50
Empty shells	4	10.25	26.75
Binucleates	14	13.14	29.93

The process of empty shell formation and nuclear doubling, and the gradual increase of uninucleates and binucleates to a condition characteristic of the original line, 150, occurred as in line 158a.

Change from binucleate to uninucleate condition (fig. 22): One peculiar departure from the normal occurred in this line. A binucleate specimen (with 14 spines and a diameter of 34 units) gave rise to a small uninucleate offspring (with 11 spines and a diameter of 28 units). The latter gave rise to a small uninucleate specimen and then formed an empty shell and became binucleate; whereas, the parent passed through empty shell formation and nuclear doubling immediately, and then gave rise to binucleate progeny, normal in diameter and spine number. The fact that empty shell formation and nuclear doubling occurred at once in the parent and was delayed in the offspring suggests that this process is initiated by the cytoplasm and takes place as soon as a sufficient quantity of cytoplasm is present. The normal size of the progeny of the parent after nuclear doubling was probably possible because of the large capacity of the shell, and indicates that the gradual increase in the size of specimens after nuclear doubling may be due, as previously suggested, to the limited capacity of the shell, rather than to the inability of the nucleus and cytoplasm to build up a sufficient quantity of protoplasm.

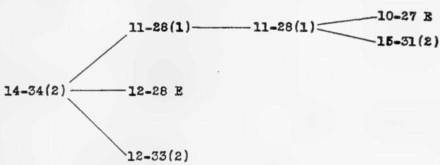


Fig. 22 Arcella dentata. Pedigree of part of the specimens reared in line 161a showing a change from the binucleate to the uninucleate condition and back again.

Experiment 4, line 162a. *Progenitor:* Uninucleate specimen from line 161a; spines, 10; diameter, 26 units (fig. 21, *C*).

Descendants: The 21 uninucleate descendants, 5 binucleate descendants, and 2 empty shells recorded in this line were similar to those described in line 158a.

Experiment 5, line 164a. *Progenitor:* Uninucleate specimen from line 162a; spines, 10; diameter, 26 units (fig. 21, *D*).

Descendants: The 10 uninucleate specimens, 5 binucleate specimens, and 3 empty shells recorded in this line were similar to those described in line 158a.

Change from binucleate to uninucleate condition: A binucleate specimen (with 12 spines and a diameter of 29 units) gave rise to a larger binucleate offspring (with 14 spines and a diameter of 31 units) and then to a large uninucleate offspring (with 16 spines and a diameter of 35 units). The parent retained only one nucleus, but immediately threw an empty shell, regained the binucleate condition, and gave rise subsequently to binucleate progeny. The large uninucleate offspring likewise immedi-

ately passed through the nuclear doubling process. This case is somewhat different from those recorded above, but the same hypotheses regarding the relation between shell size, cytoplasmic mass, and nuclear doubling may be applied successfully.

EXPERIMENT 6, LINE 165a. *Progenitor:* Uninucleate specimen from line 164a; spines, 11; diameter, 25 units (fig. 21, *E*).

Descendants: 15 uninucleates, 1 binucleate, and 2 empty shells. Specimens were kept until nuclear doubling occurred.

Discussion of experiments: The experiments described above show very clearly that there is a rather definite mass relation between the nuclei and the cytoplasm in these organisms. They also throw light upon the influence of this nucleocytoplasmic relation upon cell division, and emphasize the fact that the

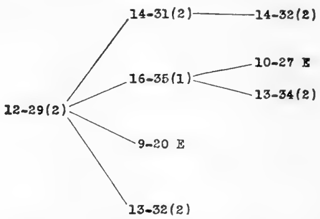


Fig. 23 Arcella dentata. Pedigree of part of the specimens reared in line 164a showing change from the binucleate to the uninucleate condition and back again.

removal of the chromidia, which were embedded in the cytoplasm that was cut away, has no effect upon the characteristics of the family. Among these characteristics are the binucleate condition, a mean spine number of about 14, and a mean diameter of about 34 units. Modifications may occur and parts of the organism may be removed generation after generation, but as long as part of a shell remains, containing a nucleus and a small quantity of cytoplasm, the line is capable of regaining its normal characteristics.

c. Experiments on members of family 152

The specimens thus far described were all members of family 150. It is interesting, however, to know if the results of removing nuclei and cytoplasm are similar in other families. The original progenitor of family 152 was collected from the pond on December 27, 1917, and like specimen 150 its spines were so minute that they could not be counted. It was 31 units in diameter. The first offspring also lacked fully developed spines; it was 32 units in diameter. This specimen was cut in two (fig. 24) and fifty descendants were reared from one of the halves. These consisted of uninucleates, binucleates, and empty shells, and these various members were interrelated just as

TABLE 6

Arcella dentata. Family 152. Table showing variations in spine number and in diameter of the uninucleates, binucleates, and empty shells belonging to family 152 (line 152.1a). The unit of measurement is 4.3 microns

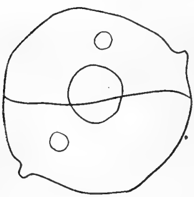
	NUMBER OF SPECIMENS	SPINE NUMBER	DIAMETER	MEAN SPINE NUMBER	MEAN DIAMETER
Uninucleates	19	7–10	22–25	9.05	23.89
Binucleates	25	10–14	26–31	11.76	29.76
Empty shells	6	8–10	22–25	9.00	23.00

were those of line 150. Table 6 gives the variations in spine number and in diameter, and the mean spine number and mean diameter of the 50 specimens in the line. The means of the binucleates are too low, since they were computed largely from the offspring of specimens that had just undergone nuclear doubling and had not yet reached the size characteristic of the race.

The experiments on members of this family thus confirm the results derived from a study of family 150, and demonstrate that the processes described in the latter are similar in families derived from different wild specimens.

d. Experiments on members of family 58

1. Line 153. The progenitor of this line was a member of the high line of family 58 which was being used for selection work when this series of experiments was begun (January 10, 1918). The specimen operated upon had 12 spines and was 28 units in diameter (fig. 25), and the line from which it was taken had at the same time a mean spine number of 11.48 and a mean diameter of 27.26 units. The pedigree shown in figure 26 gives part of the history of this line. The half of the parent



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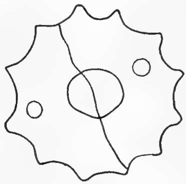

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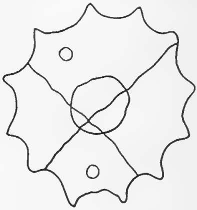

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Fig. 24 Arcella dentata. Outline of the first offspring of the progenitor of family 152. The cross line indicates where the specimen was bisected. × 207.

Fig. 25 Arcella dentata. Outline of a specimen of family 58 which served as the progenitor of line 153. The cross line shows where the specimen was bisected. × 207.

Fig. 27 Arcella dentata. Outline of the progenitor of line 155, showing where it was cut into four pieces. × 207.

labeled 153a threw an empty shell immediately and the rest of its offspring and other descendants were all binucleate. The half labeled 153b doubled after producing three offspring. Nuclear doubling throughout this entire line occurred earlier than in the lines already described, but there is no apparent reason for this behavior and it is probably of no significance. The data are presented in compact form in table 7. The mean spine number and mean diameter of the binucleates is lower than that of the line from which they were descended because the small specimens that were produced immediately after nuclear doubling are included. Many of these had only 8 or

9 spines and were only from 21 to 25 units in diameter, whereas some of the last specimens in the pedigree had spine numbers of 11, 12, and 13 and diameters of 27, 28, and 29 units.

Line 153 resembles the lines already described in the relations of the uninucleate, binucleate, and empty specimens, and in the nuclear doubling process.

2. Line 155. The progenitor of this line was 29 units in diameter and possessed 12 spines.. It was taken from the same line as specimen 153, but was cut into four pieces, as shown in figure 27. The larger nucleated piece was labeled 155a and

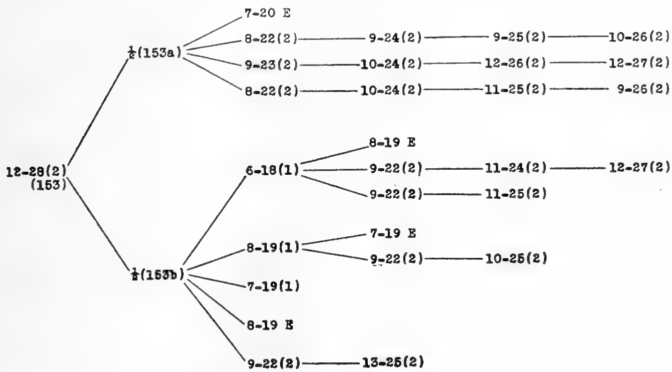

Fig. 26 Arcella dentata. Pedigree of part of the specimens in line 153

the smaller, 155b. The pieces without nuclei died in a short time. The pedigrees of the two lines derived from 155a and 155b are in part shown in figure 28. As in lines 150.2a and 150.2b,

TABLE 7

Arcella dentata. Family 153. Table showing variations in spine number and in diameter, of the uninucleates, binucleates, and empty shells belonging to family 153. The unit of measurement is 4.3 μ

	NUMBER OF SPECIMENS	SPINE NUMBER	DIAMETER	MEAN SPINE NUMBER	MEAN DIAMETER
Uninucleates	6	6–9	18–19	7.50	18.83
Binucleates	46	8–13	22–29	10.33	24.78
Empty shells	5	7–8	19–20	7.40	19.20

the descendants of the smaller piece (155b) were at first smaller and possessed fewer spines than those of the larger piece (155a), but after several generations they became similar in these characters. The formation of empty shells and nuclear doubling occurred as in the other lines studied, and the specimens after becoming binucleate, soon regained the mean condition of the line from which the original progenitor of the line was taken. Figure 29 shows stages from one original quarter of specimen 155 to the attainment of the normal binucleate condition. The line was discontinued when specimens with 11 and 12

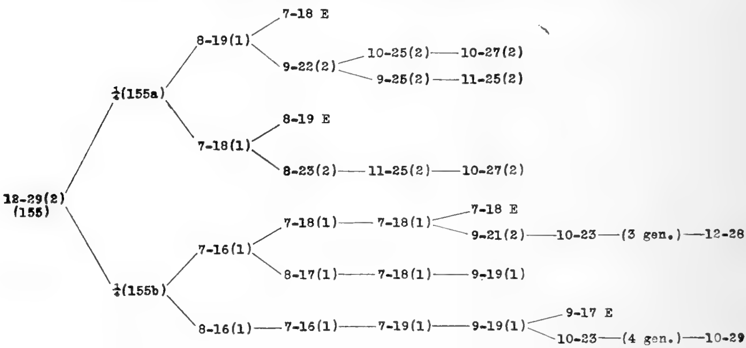

Fig. 28 Arcella dentata. Pedigree of part of specimens in line 155

spines and with diameters of from 27 to 29 units began to appear. Table 8 contains the data as regards spine number and diameter in the members of the line. It is thus evident that one nucleus and less than one-fourth of the normal amount

TABLE 8

Arcella dentata. Family 155. Table showing variations in spine number and in diameter of the uninucleates, binucleates, and empty shells belonging to family 155. The unit of measurement is 4.3 μ

	NUMBER OF SPECIMENS	SPINE NUMBER	DIAMETER	MEAN SPINE NUMBER	MEAN DIAMETER
Uninucleates	26	6–9	15–21	7.74	17.68
Binucleates	29	8–12	21–29	9.93	25.17
Empty shells	4	7–9	17–19	7.75	18.00

of cytoplasm will in a few generations give rise to specimens with the nuclear condition and shell characteristics of the line.

3. Lines 156 and 157. These two lines were derived from two specimens taken from the stock being used for selection work in family 58. Fifteen specimens were reared in line 156 and 22 specimens in line 157. In both lines the relations between uninucleates, binucleates, empty shells, and the process of nuclear doubling were similar to those observed in the other lines described above.

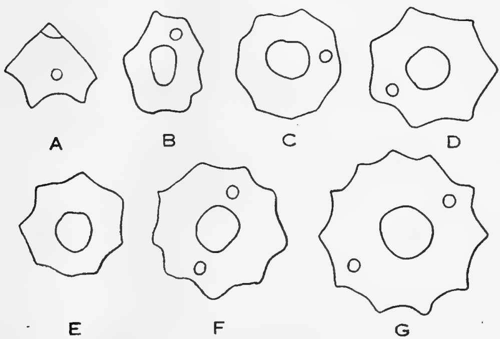

Fig. 29 Arcella dentata. Specimens selected from line 155b to show the stages from the original progenitor to the attainment of the normal binucleate condition. *A* = original progenitor (155b). *B* = first offspring of *A* (155b1). *C* = first offspring of *B* (155b1.1). *D* = first offspring of *C* (155b1.1.1.). *E* = first offspring of *D* (155b.1.1.1.1). This was empty. *F* = second offspring of *D* (155b.1.1.1.2). This was binucleate. *G* = normal binucleate, being the great granddaughter of *F* (153b.1.1.1.2.1.1.1). × 207.

e. Summary of results of experiments on Arcella dentata

1. When part of the shell is removed there is no regeneration of the missing piece.

2. When part of the cytoplasm is removed the f_1 progeny are smaller than the parent, but the parental size is regained in the succeeding generation, thus showing that the size of the offspring depends in part upon the amount of cytoplasm within the parent, and possibly also upon the capacity of the shell

regardless of the character of the parental nuclei, but that the mass relations between the nuclei and cytoplasm are such as to lead to an equilibrium when a condition 'normal' for the line is reached.

3. Part of the chromidial net was also removed with the cytoplasm. The elimination of chromidia, however, has no apparent effect upon the characteristics of the offspring.

4. When specimens are cut in two, each uninucleate piece continues to live, and reproduces as rapidly as before the operation. The offspring, which are uninucleate, are slightly irregular in shape in the f_1 generation, but of normal shape in succeeding generations.

5. The descendants of large uninucleate pieces are at first larger than those of smaller uninucleate pieces. This indicates that the amount of cytoplasm within the parent influences the size of the offspring. The quantity of cytoplasm is probably, in turn, dependent upon the capacity of the shell. Within a few generations, however, descendants of unequal pieces of the same original parent are approximately equal in size, showing that there is a definite normal size for the uninucleates in this family. The establishment of this normal size appears to be due to the interaction of the nucleus and cytoplasm resulting in a gradual increase in the quantity of the latter until an equilibrium between the two is attained. Since, therefore, the nuclei of the two original pieces were alike, the members of the two lines derived from them finally reached similar proportions.

6. Uninucleate specimens become binucleate after a varying number of immediate offspring and of generations have been produced. The uninucleate parents become binucleate during a process of empty shell formation. The parent secretes a new shell, usually smaller than itself, but the nucleus and cytoplasm destined for this shell are withheld by the parent and the shell is cast off in an empty condition. The parent thus retains two nuclei and all of the cytoplasm.

7. Specimens that have just become binucleate produce binucleate offspring slightly larger than themselves. These offspring, in turn, produce still larger progeny until after three

or four generations the diameter and spine number of the original line before the cutting operation was performed are regained. This procedure proves that in these organisms a marked change in the condition of the germ plasm (the change from the uninucleate to the binucleate state) is not immediately expressed in full by the somatoplasm, but that several generations are required for complete adjustment. As in the case of the uninucleates described in paragraph 5, the gradual assumption of the normal characteristics of the binucleates of the family is probably due to the fact that the capacity of the shell limits the quantity of cytoplasm and hence prevents the attainment of the complete size in one generation.

8. Empty shells were produced by several binucleate specimens, but this process did not lead to an increase in the number of nuclei, and no modifications of the cytoplasm or shell characteristics were noted in the later offspring.

9. Diameter and spine number are closely correlated in both binucleates and uninucleates; the coefficient for the binucleates is 0.640 ± 0.027 and that for the uninucleates 0.402 ± 0.039.

10. In several cases changes from the binucleate to the uninucleate condition occurred. The uninucleate parents and progeny later regained the binucleate condition during empty shell formation. Hypotheses are presented to account for these changes.

11. Nucleated pieces of bisected uninucleate specimens continue to live and reproduce, giving rise to offspring smaller than the original parent. These offspring produce larger uninucleate specimens and these in turn form larger offspring until after three or four generations the normal diameter and spine number of the uninucleates of the family are regained.

12. The bisection of uninucleates belonging to five succeeding lines had no permanent influence upon the characteristics of the family, since in every case the normal characteristics of the uninucleates were regained; empty shell formation occurred, and binucleates like those of the original line were finally produced. In one line no empty shells nor binucleates were recorded, but this was no doubt because the line was not kept long enough.

13. A uninucleate-half of a specimen was formed by a normal binucleate, which later gave rise to normal binucleate offspring. This uninucleate-half produced uninucleate offspring which passed through empty shell formation and nuclear doubling just as did those derived from uninucleate-halves obtained by cutting. When a uninucleate from this line was bisected, the nucleated piece behaved as did those described in paragraphs 11 and 12. It is probable that pieces resulting from the action of mechanical factors that occur in nature react in a manner similar to those obtained in the laboratory by means of operations. The race is thus not permanently affected by accidental mutilations of its members.

14. The removal of one nucleus from binucleate specimens results in uninucleate specimens containing an amount of cytoplasm larger than that usually associated with one nucleus. In most cases such specimens form empty shells and become binucleate at once. When uninucleate offspring are produced, these are larger than those formed by uninucleate parents with only half of the cytoplasm of the original parent, and they undergo empty shell formation and nuclear doubling more quickly than the latter. This is probably due to the inequality between the single nucleus and the large cytoplasmic mass, and suggests that the process of nuclear doubling is initiated when the cytoplasmic mass becomes greater than the quantity normally associated with one nucleus.

15. Operations similar to those summarized in the above paragraphs were performed on specimens belonging to two other families with the same results. In one case (line 155) a specimen was cut into four pieces, and the descendants of the two nucleated quarters eventually regained the characteristics of the original line.

3. OBSERVATIONS AND EXPERIMENTS ON ARCELLA POLYPORA

a. Origin of the specimens studied

The studies of these multinucleate Arcellas (fig. 30) were carried on at Johns Hopkins University from March 16 to June 16, 1918, and continued at Cold Spring Harbor, Long Island, from June 17th to August 28th.

The first specimens were found in a flower pot containing floating leaves of Azolla caroliniana, in the greenhouse on the university campus. Others were later discovered in a pond

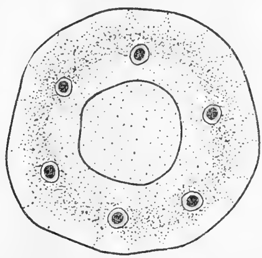

Fig. 30 Arcella polypora. Drawing showing the structure of a typical specimen. × 310.

on the campus and in ponds at Cold Spring Harbor. These Arcellas resembled descriptions of A. discoides, but on examination were found to possess more than two nuclei.

b. Relations between nuclear number and diameter in wild specimens

Twenty specimens were fixed and stained on April 1st and their diameters and nuclear numbers recorded. Thirty-four more were measured and their nuclei counted in the living state on June 19th. Table 9 shows the distribution of sizes and nuclear numbers in these fifty-four specimens. Other specimens were collected at this time that measure 23, 24, 34, and 35 units in diameter.

TABLE 9

Arcella polypora. Correlation table for number of nuclei and diameter of shell in fifty-four specimens collected at Baltimore. The unit of measurement is 4.3 μ. Coefficient of correlation 0.156 ± 0.089

DIAMETER OF SHELL	NUMBER OF NUCLEI								
	3	4	5	6	7	8	9	10	
25	1	2	4		2				9
26				2				1	3
27		1	2	1					4
28			5	2	2	1			10
29			7	4		3		1	15
30		1	2	2					5
31		1				1			2
32		1	1		1	1			4
33				1	1				2
Totals...	1	6	21	12	6	6		2	54

c. Variations in diameter within families

Thirty-three specimens were isolated on culture slides and from these twenty-five small families and one large family were reared. The most striking difference between these families and those reared from specimens of A. dentata was the great variations in size that often occurred between parent and offspring. The following are examples of some of these differences:

DIAMETER OF PARENT	DIAMETER OF OFFSPRING
34	26
34	24
31	26
31	20
29	24
27	21
26	20
25	29
25	30
25	32
22	26

Part of the pedigree of family 22 (fig. 31) shows several of these large variations in diameter.

d. Relations between nuclear number and diameter within small families and between families

The nuclei were counted in only a few of the specimens at this time, but it was soon discovered that their number varied in the members of a single family, and that one branch of a family might consist of specimens with a certain number of nuclei and another branch of the same family of specimens with a different number. Thus in family 11 there was one branch with 5 nuclei and another with 6; in family 26, one with 7 and another with 8; in family 30, one with 6 and another with 7, and in family 12, one with 4 and another with 6.

It was also noticed that the specimens belonging to a branch containing the lesser number of nuclei tended to be smaller

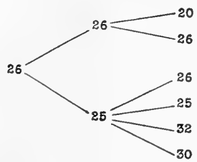

Fig. 31 Arcella polypora. Pedigree of part of the specimens in family 22 showing large variations in diameter. The unit of measurement is 4.3 μ.

than those belonging to another branch of the same family with more nuclei. However, the members of a branch of one family with a certain number of nuclei, differed in their diameter from the members of branches of other families with the same number of nuclei. For example, in family 11 the measurements of the nine specimens in the 5-nucleated branch were as follows: 31, 30, 30, 29, 29, 28, 28, 28, 28, whereas in family 6 the five specimens in the 5-nucleated branch measured 23, 24, 25, 25, and 26 units.

Table 10 gives the distribution of diameters and the mean diameters of thirteen families. The numbers in each family are small, but it seems probable that the different families differ constitutionally in diameter. This appears particularly when families 6 and 7 are contrasted with families 8, 11, 12,

and 29. This result is what the studies of various investigators of pure lines and my own studies of A. dentata would lead one to expect.

Table 11 gives the correlation in diameter of parents and offspring within the twenty-five small families.

e. Relations between nuclear number and diameter within a large family

Family ap. 5. The members of family ap. 5 were more viable and multiplied more rapidly than those in the other families, hence this line was chosen for further investigation. Nuclei were counted only occasionally during the first two periods described below; they varied in number from 5 to 7.

1. Correlation in diameter between parents and immediate offspring. Period 1. This period extended from March 18 to May 10, 1918. The 173 parents and offspring are recorded in correlation table 12, and the first part of the pedigree is presented in figure 32.

2. Results of selection for lines with large and small diameters. Period 2. On May 11th selection for diameter was begun on the basis of past performance, and the following specimens were chosen as progenitors of the high and low lines, respectively.

HIGH LINE		LOW LINE	
Number	Diameter	Number	Diameter
1132	32	1121	27
11321	31	11213	27
11322	30	112131	22
113211	31	11214	21
		112141	22
		11215	22

All parents and offspring within the high line with a diameter of from 28 to 32 units were kept unless they persisted in producing young with a diameter less than 28; and likewise in the low line, all parents and offspring with a diameter of 24 units or less were retained unless they habitually gave rise to progeny

TABLE 10

Arcella polypora. Table showing the distribution of the diameters of the shells within thirteen families during the first period (March 18 to May 10, 1918). The unit of measurement is 4.3 μ

NUMBER OF FAMILY	DIAMETER OF SHELL																			NUMBER OF SPECIMENS	MEAN DIAMETER
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35		
5						4	1	10	17	22	13	23	32	45	4	2				173	27.79
6	1	1		3	5	4	2	3	4	3	1							1		28	23.03
7			1		2	1		2	2	3	3	2								16	24.75
8										2	1	1		5	7	3				19	30.00
10					2	1	2	2	2	4	7	1								21	25.19
11											1	7	5	3	3	1				20	29.15
12									2		1	2	8	10	8	5				36	29.80
13							1	2	6	9	7	1								26	25.84
14				1			1		3	1	6	2			1					15	26.20
15									2	7	3	1	1							14	26.42
16								1	4	3	5	6						1		20	26.95
22				1				2	3	4				1		1				12	25.75
29												1	3	2	1	3			1	11	30.63

TABLE 11

Arcella polypora. Correlation table for diameter of the shell in parents and progeny within twenty-five small families. The unit of measurement is 4.3 μ. Coefficient of correlation, 0.740 ± 0.018

PROGENY	PARENTS																
	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	
17	1																1
18		1															1
19		1															1
20	1		2				1					1					5
21	1	3	1	1	1		1	2									10
22	1	2	1		1		2										7
23	1		1	1	3	1		1									8
24	1	5		1	1	1	2		1	1					1		14
25			1	4	2	6	6	7	2	1	1	1					31
26			2		5	8	8	9	1			3			1		37
27					2	7	8	8	5	3	1	1		1	1		37
28						1	3	8	4	3	4	2	2				27
29						1		3		7	7	2	4		1		25
30									1	2	6	7	6	1	4	1	28
31										3	9	4	6		1	2	25
32						1				1	2	4	2			4	14
Totals...........	6	12	8	7	15	26	31	38	14	21	30	25	20	2	9	7	271

larger than 24 units. After several generations it became necessary to raise the upper limit in the low line from 24 to 25 and then to 26 units, because of the small number of specimens produced with a diameter less than 25 units. An attempt was made to count the nuclei of the specimens obtained during this period, and in a large proportion of the cases this was accom-

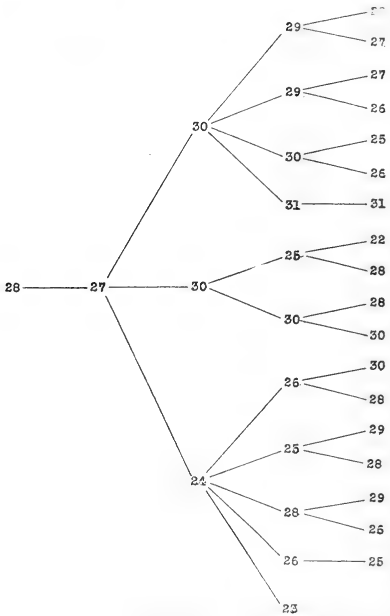

Fig. 32 Arcella polypora. Family ap. 5. Pedigree of part of the specimens obtained during the first period. The variations in diameter are large.

plished. The data obtained are presented in tables 13 and 14. As indicated in table 13, the mean difference in diameter between the high and low lines was 4.75 units. This difference is very much greater than it was possible to obtain with A. dentata during a longer period with many more specimens and a larger number of generations.

3. Relations between nuclear number and diameter during the selection period. The difference between the efficacy of selection in the two species can easily be accounted for on the assumption that there is a quantitative relation between the nuclei and the cytoplasm associated with them. In A. dentata all but a negligible number of the specimens used in the selection experiments possessed two nuclei. In A. polypora, on the other hand, all of the specimens in the low line that were measured

TABLE 12

Arcella polypora. Family ap. 5. Correlation table for diameter of the shell in parents and progeny during the first period of fifty-four days when no selection was practiced. The unit of measurement is 4.3 μ. Coefficient of correlation, 0.442 ± 0.041

PROGENY	PARENTS											
	22	23	24	25	26	27	28	29	30	31	32	
22	1			1								2
23			3	2	2	1	1					9
24	1	1		4	3	3		2				14
25			2	1	4	1	2	3	3		1	17
26	2		4	2	2	1	5	5	3	1		25
27				2	3	1	4	8	6			24
28			1	2	5		4	6	8			26
29				2	1		4	4	9	2		22
30				1	2	6	3	4	11			27
31									3	1		4
32									2		1	3
Totals....	4	1	10	17	22	13	23	32	45	4	2	173

were provided with 4 nuclei, whereas all those in the high line that were measured contained 5 or 6 nuclei. The conclusion that these facts suggest is that the members of the low line are smaller than those of the high line because of their lesser number of nuclei. They also indicate that the frequent large differences in diameter between parent and offspring may be due to a change in nuclear number, that is, when the offspring was much larger than the parent an increase in nuclear number probably occurred, and when the offspring was much smaller, a decrease in nuclear number was responsible. No very great

changes in size took place during this period, but later work proved this explanation to be correct. The correlation between the parents and offspring during this period is very striking (table 14).

TABLE 13

Arcella polypora. Family ap. 5. First selection period. Table giving the mean diameter of the shell and the number of nuclei possessed by the specimens in the high line (ap. 5a) and low line (ap. 5b). The unit of measurement is 4.3 μ

LINE	NUMBER OF SPECIMENS[1]	MEAN DIAMETER	NUMBER WITH 4 NUCLEI	NUMBER WITH 5 NUCLEI	NUMBER WITH 6 NUCLEI
High (ap. 5a)..................	96	29.72		24	65
Low (ap. 5b)..................	41	24.97	40		

[1] The number of nuclei could not be determined with certainty in a few of the specimens.

TABLE 14

Arcella polypora. Family ap. 5. Correlation table for diameter of the shell in parents and progeny during a period of eight days when selection for specimens with large and small diameters was practiced. Coefficient of correlation, 0.742 ± 0.258

PROGENY	PARENTS												
	21	22	23	24	25	26	27	28	29	30	31	32	
21							1						1
22	1						4						5
23	1	3											4
24	1	2	2	2			1						8
25	1	4	2	1			2						10
26		1		2		1							4
27			1	2	1		1			1			6
28					2		1	3		6	2		14
29							2	1	5	11	7	1	27
30								4	1	14	15	3	37
31										6	5	6	17
32									2		2		4
Totals...........	4	10	5	7	3	1	12	8	8	38	31	10	137

4. Results of selection for large and small diameters within groups with the same number of nuclei. Period 3. On May 19 a new selection period of twenty-four days was inaugurated.

During this time the specimens were separated into classes according to the number of nuclei they possessed, and both the largest and the smallest members were saved in each class, all others being discarded. Here, as usual, past performance was used as a basis for selection. Table 15 shows the results obtained. The specimens fell into four classes with 3, 4, 5, and 6 nuclei, respectively. The range of variation in diameter was

TABLE 15

Arcella polypora. Family ap. 5. Correlation table for nuclear number and diameter of the shell in 263 specimens. These were obtained during a period of twenty-four days when the largest and smallest specimens under each nuclear number were selected. The unit of measurement is 4.3 μ. Coefficient of correlation, 0.752 ± 0.018

DIAMETER OF SHELL	NUMBER OF NUCLEI				
	3	4	5	6	
22	2	1			3
23	5	2			7
24	19	2			21
25	15	6	2	1	24
26	13	11	2	3	29
27	6	15	13	2	36
28	3	15	20	4	42
29	1	1	23	22	47
30	1		15	24	40
31			4	10	14
Totals........	65	53	79	66	263

about the same for those with 3 and 4 nuclei, and exactly the same for those with 5 and 6 nuclei. The mean diameter, however, increased steadily from that of 25.12 units in the 3-nucleated class to 29.35 units in the 6-nucleated class. The coefficient of correlation between nuclear number and diameter is large and, of course, would have been much greater if selection had not been practiced. Figure 33 shows by means of curves the differences in diameter of the groups in family ap. 5 arranged according to nuclear number.

5. Changes in nuclear number and their results. The changes in nuclear number during this period were of two principal

types. Most of the changes consisted in an increase or a decrease of one nucleus, but in a few cases a doubling of the nuclear number from 3 to 6 occurred. Thus the specimen labeled ap. 5. b. a. 1. 1. 1. 1. 1. was 27 units in diameter and possessed 3 nuclei. On May 27th it gave rise to an offspring that was 29 units in diameter and possessed 6 nuclei. The parent, however, retained the original number, 3. Six later offspring of this parent all contained 3 nuclei and had a mean diameter of 24.33 units, whereas the three descendants of the 6-nucleated offspring all possessed 6 nuclei and had a mean diameter of 29.50

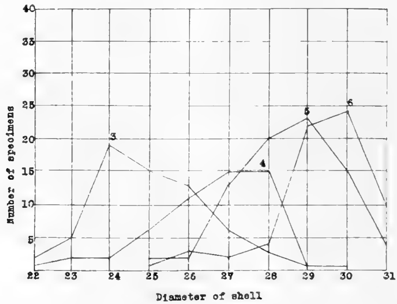

Fig. 33 Arcella polypora. Family ap. 5. Curves for the variations in diameter in groups of specimens arranged according to nuclear number; plotted from table 15. The ordinates are numbers of specimens; the abscissae are diameters, and the numbers at the top of the curves indicate the number of nuclei in the corresponding groups of specimens.

units. The difference of 5.17 units in the mean diameter of these two groups is obviously due to the difference in nuclear number.

In another case a specimen (ap. 5 b. a. 1. 1. 4) 22 units in diameter and with 3 nuclei gave rise to four progeny. Three of these had 3 nuclei and a mean diameter of 24.33; the fourth was 27 units in diameter and possessed 6 nuclei. The three descendants of the latter had 6 nuclei and a mean diameter of 28 units. The differences were not as great when they

involved a decrease or increase of only one nucleus, but they were nevertheless easily observed and very constant. Some of these are indicated in table 16.

6. Results of a further increase in nuclear number. Period 4. On June 17, 1918, specimens were transferred to the laboratory at Cold Spring Harbor, Long Island. They were not affected by the change of food thus made necessary, and continued to divide about every two days. Two principal problems were attacked during this period: 1) an attempt was made to obtain specimens in family 5 with more than 6 and less than 3 nuclei, and, 2) new families were reared (from wild specimens brought

TABLE 16

Arcella polypora. Family ap. 5. Table showing the difference in diameter of parent and offspring at the time of changes in the number of nuclei. The unit of measurement is 4.3 μ

DIAMETER OF PARENT	NUMBER OF NUCLEI OF PARENT	DIAMETER OF OFFSPRING	NUMBER OF NUCLEI OF OFFSPRING	DIFFERENCE IN NUMBER OF NUCLEI	DIFFERENCE IN DIAMETER
32	5	26	3	2	6
30	5	29	4	1	1
31	6	30	5	1	1
31	6	29	5	1	2
30	6	29	5	1	1
30	6	29	5	1	1
29	6	29	5	1	0

from Baltimore) with the object of determining what differences exist between different families with respect to nuclear number and the diameter of the shell.

Specimens in family 5 with 7 nuclei. Table 17 gives the correlation between the number of nuclei and the diameter of the shell of ninety-two specimens that were reared at Cold Spring Harbor from June 17 to July 27, 1918. Here again as in those reared at Baltimore (table 15) there is a high correlation between these two characters. The mean diameter in each class differs somewhat from that of the corresponding class obtained at Baltimore, but this is probably due to the small number of specimens involved. In both tables (15 and 17) the mean diameter increases as the nuclear number increases.

Of particular interest are the thirty-two specimens in table 17 in the 7-nucleated class. These were all derived from a single progenitor with 7 nuclei. The parent of this progenitor had 4 nuclei and gave rise to three other progeny, two of which possessed 4 nuclei and the third, 6. Among the progeny of the 7-nucleated specimens were 4 with 6 nuclei and one with 5. The greater mean diameter of the 7-nucleated specimens may be accounted for by the presence of one more nucleus than in the specimens previously studied.

TABLE 17

Arcella polypora. Family ap. 5. Correlation table for nuclear number and diameter of shell of ninety-two specimens reared at Cold Spring Harbor. The unit of measurement is 4.3 μ. Coefficient of correlation, 0.818 ± 0.023

DIAMETER OF SHELL	NUMBER OF NUCLEI					
	3	4	5	6	7	
22		1				1
23						
24	2	1				3
25	2	3	1			6
26	4	4	2			10
27	2	4	3			9
28		2	2	4	2	10
29		1	3	4	5	13
30				11	13	24
31				3	8	11
32				1	4	5
Totals.......	10	16	11	23	32	92

7. *Uninucleate and binucleate specimens obtained by cutting experiments.* Since no specimens were found with only one or two nuclei, cutting experiments were resorted to to obtain them. These experiments were carried on in the same way as those performed earlier on A. dentata and described in part 2 of this paper. Ten specimens were operated on in this series of experiments. These specimens possessed 3, 4, or 5 nuclei, and were cut in two so that the pieces contained 1, 2, or 3 nuclei each (fig. 34). From these pieces nine lines were obtained. Some of the data derived from these lines are indicated in table

18. In most cases the nuclear number of the offspring differed from that of the parental piece, and the diameter and nuclear number of the original parent was very quickly regained. Several of the families, however, were discarded before this was fully accomplished.

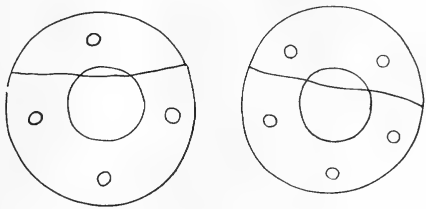

Fig. 34 Arcella polypora. Outlines of specimens showing where they were cut in two. The uninucleate part of *A* became the progenitor of line apc. 7 and the binucleate part of *B* became the progenitor of line apc. 9. × 207.

TABLE 18

Arcella polypora. Family ap. 5. Table showing the number of nuclei and diameter of original parents used in the cutting experiments; the number of nuclei in the pieces; and the number of nuclei in and the diameter of the offspring of the pieces. The unit of measurement is 4.3 μ

NUMBER OF LINE	ORIGINAL PARENT		NUMBER OF NUCLEI IN PIECE	FIRST OFFSPRING		SECOND OFFSPRING		THIRD OFFSPRING	
	Diameter	Number of nuclei		Diameter	Number of nuclei	Diameter	Number of nuclei	Diameter	Number of nuclei
Apc 1	26	5	4	21	3	22	4		
Apc 3	27	3	2	27	3	28	5	26	5
Apc 4	28	4	2	21	2				
Apc 5	28	4	2	18	3	23	4		
Apc 6	28	4	3	24	4	27	4	25	4
Apc 7	28	4	1	19	1	17	1	19	2
Apc 9	27	5	3	20	2	19	2	20	2
Apc 14	26	3	2	20	1	20	1	21	3
Apc 16	29	4	2	20	2				

Two of the lines are of particular interest, since many specimens with 1 or 2 nuclei were obtained from them. Data for the specimens in line apc. 7 are given in table 19 and part of the pedigree of this line is shown in figure 35. The one empty shell produced is not included in the table. The mean diameter of the classes with 3, 4, 5, or 6 nuclei is less than that shown

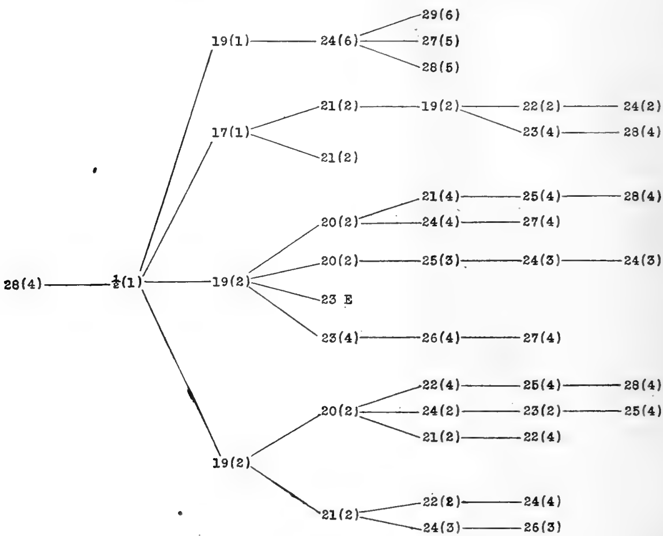

Fig. 35 Arcella polypora. Family apc. 7. Pedigree showing diameters, number of nuclei, and an empty shell.

in table 15, because specimens were included in each class before the full size normal for that particular number of nuclei had been attained. The class to which attention should be directed in particular is that with 2 nuclei. In this class there were four specimens with a diameter of 19 units; three of 20; four of 21; two of 22; one of 23, and three of 24. The three that were 24 units in diameter and the one of 23 units were peculiar, since their nuclei were much larger than those within the other binu-

TABLE 19

Arcella polypora. Family ap. 5, line apc. 7. Table showing the mean diameter of specimens with different nuclear numbers within a line derived from a uninucleate piece of a specimen with 4 nuclei and a diameter of 28 units. The unit of measurement is 4.3 μ

NUMBER OF NUCLEI	NUMBER OF SPECIMENS	MEAN DIAMETER
1	1	17
2	17	21.12
3	9	24.67
4	26	24.92
5	6	28.17
6	2	26.50

cleate specimens. All four of them immediately underwent nuclear doubling, and they and their offspring then each possessed 4 nuclei.

Line apc. 9 is interesting because the binucleate condition was here maintained for a period of thirty-eight days (June 21st to July 29th), during which nine generations were obtained without the intervention of a nuclear change. Seventeen specimens were recorded during this period, the last of which possessed 4 nuclei and all the others 2. The diameter of the sixteen binucleate specimens ranged from 15 to 22 units with a mean of 18.80. One empty shell was produced in this line. From these data it appears that a binucleate line is not easily obtained and will not persist for any great length of time.

In table 20 is given the distribution of the diameters of the specimens with 1 and 2 nuclei in the nine lines in this series. The mean diameters of 19 units for the uninucleate class and of

TABLE 20

Arcella polypora. Family ap. 5. Table showing the distribution of the diameters of the shells of specimens with 1 and 2 nuclei. The unit of measurement is 4.3 μ

NUMBER OF NUCLEI	DIAMETER OF SHELL										NUMBER OF SPECIMENS	MEAN DIAMETER
	15	16	17	18	19	20	21	22	23	24		
1			1		1	2					4	19.00
2	1			6	6	8	5	3	1	2	32	19.97

19.97 units for the binucleate class are probably low, since specimens are included in the table that had no doubt not yet reached the full size characteristic of 1 or 2 nuclei. When the means for the various classes given in tables 15, 17, and 20 are combined and averages obtained, the following data result:

NUMBER OF NUCLEI	NUMBER OF SPECIMENS	AVERAGE MEAN DIAMETER
1	4	19.00
2	32	19.97
3	75	25.36
4	69	26.36
5	90	28.00
6	89	29.52
7	32	30.22

f. Differences between different families with regard to nuclear number and diameter

1. Variations in nuclear number and diameter in wild specimens and within small families. As noted on page 36, considerable variation between diameter and nuclear number was found among wild specimens. Some of these are as follows:

DIAMETER	NUMBER OF NUCLEI
26	10
31	8
28	8
25	7
32	7
26	6
33	6
25	5
32	5
25	4
32	4

Also among the twenty-five small families there were some with a large number of nuclei that were smaller in diameter than others with fewer nuclei. For example, in family 12 twenty-three specimens with 6 nuclei averaged 30.43 units in diameter

and in family 16 nineteen specimens with 7 nuclei had a mean diameter of 26.58 units. The distribution of diameters in these two families was as follows:

	DIAMETER								
	24	25	26	27	28	29	30	31	32
Number of specimens in family 12						4	9	6	4
Number of specimens in family 16	1	4	3	5	6				

2. Differences between family ap. 5 and families ap. 38, ap. 39, ap. 69 and ap. 34. All of the small families were eliminated early in the course of the work, in order that more attention could be directed to family ap. 5; but on June 19th a new set of wild specimens were isolated from the same material from which the others were obtained and eight new families were reared. Five of these were stopped after a short period, the other three were carried along for a few days, and finally only one family was kept for further work. The two smaller families were labeled ap. 38 and ap. 39.

a. Families ap. 38, ap. 39, and ap. 69. Of the thirty-eight specimens in family ap. 38, thirty-one possessed 8 nuclei each, one had 7, and the other six had 6. There were twenty-six specimens in family ap. 39, all with 7 nuclei except four which possessed 6 each. The distribution of diameters of the 8-nucleated specimens in family ap. 38 and the 7-nucleated specimens in family ap. 39 is as follows:

	DIAMETER					
	24	25	26	27	28	Mean diameter
Specimens of family ap. 38 with 8 nuclei	4	4	7	9	7	27.26
Specimens of family ap. 39 with 7 nuclei	3	6	6	4	3	25.91

There is a striking difference between the mean diameters of these families and the mean diameter of family ap. 5 when considered from the standpoint of nuclear number. In family ap. 5 specimens with 7 nuclei had a mean diameter of 30.22 units as contrasted with 25.91 in family ap. 39.

Family ap. 69 was started on June 29th with a specimen collected at Cold Spring Harbor. Its nine members all possessed 6 nuclei and ranged in diameter from 32 to 36 units with a mean diameter of 34.22 units. This family thus differs from both family ap. 5 and from families ap. 38 and ap. 39, its members being somewhat larger than those of family ap. 5 in comparison to the number of nuclei present, and very much larger in this respect than the members of families ap. 38 and ap. 39. These data make more certain the conclusion reached as the result of

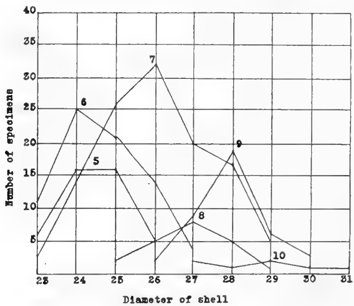

Fig. 36 Arcella polypora. Family ap. 34. Curves for the variations in diameter in groups of specimens arranged according to nuclear number; plotted from table 21. The ordinates are numbers of specimens; the abscissae are diameters, and the numbers at the tops of the curves indicate the number of nuclei in the corresponding groups of specimens.

earlier observations, that different families differ with regard to nuclear number and diameter.

b. Family ap. 34. Comparison of nuclear number and diameter with family ap. 5. Family ap. 34 was chosen for extended observations, and records were made of 355 specimens. The distribution of the diameters of 302 of these is shown in table 21 and also the correlation between nuclear number and diameter within the family. The nuclei could not be counted with certainty in fifty-three of the specimens, and hence these are not included in the table. Figure 36 shows by means of curves the differences in diameter of the groups in family ap. 34 arranged

according to nuclear number. The coefficient of correlation between nuclear number and diameter within family ap. 34 is very similar to that within family ap. 5 (table 15), but in the former a particular number of nuclei is on the average associated with a diameter about 4 units smaller than within family ap. 5. Furthermore, the variation in the number of nuclei is different, since in family ap. 5 specimens with from 3 to 7 nuclei were obtained without recourse to cutting experiments, whereas in family ap. 34 the range was from 5 to 10. Figure 37 shows by means of

TABLE 21

Arcella polypora. Family ap. 34. Correlation table for number of nuclei and diameter of shell in 302 specimens. The unit of measurement is 4.3 μ. Coefficient of correlation, 0.699 ± 0.020

DIAMETER OF SHELL	NUMBER OF NUCLEI						
	5	6	7	8	9	10	
23	6	11	3				20
24	16	25	14				55
25	16	21	26	2			65
26	5	14	32	5	2		58
27		4	20	8	9	2	43
28			17	5	19	1	42
29			5	1	6	2	14
30					3	1	4
31						1	1
Totals....	43	75	117	21	39	7	302

curves the similarity in diameter of the group in family ap. 5 with 4 nuclei and of the group in family ap. 34 with 7 nuclei.

Diverse lines with 7 nuclei within family ap. 34. During the course of the work with family ap. 34 it was noticed that several of the branches that contained mostly specimens with 7 nuclei, differed considerably in size. Members of these branches were kept for eight generations and during the entire period of twenty days covered by these generations the difference in diameter persisted (fig. 38). The distribution according to diameters of these two lines was as follows:

	Diameter							Total number	Mean
	23	24	25	26	27	28	29		
Small line	2	10	1	1				14	24.07
Large line			1	4	7	8	3	23	27.35
Difference									3.28

While these numbers are too small for definite conclusions, it appears very probable that lines permanently diverse in diameter but with the same number of nuclei might easily be isolated within this family.

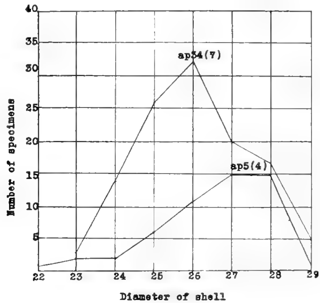

Fig. 37 Arcella polypora. Curves for the variations in diameter in the group of specimens of family ap. 5 with 4 nuclei and of family ap. 34 with 7 nuclei; plotted from tables 15 and 21. The ordinates are numbers of specimens; the abscissae are diameters, and the numbers at the top of the curves indicate the number of nuclei in the corresponding group of specimens.

g. Relation between diameter of shell and diameter of mouth in wild populations and within families

Among the specimens of Arcella collected at Cold Spring Harbor were a number of multinucleates that were much greater in diameter than any previously discovered and in which the mouth opening obviously was larger in comparison with the diameter of the shell than was true of the other multinucleate specimens examined. These specimens ranged in diameter from 176 μ to 232 μ, whereas the other multinucleates under observation ranged

in diameter from 73 μ to 163 μ. No differences other than the large diameter of the shell and of the mouth were observed between these large multinucleates and the smaller specimens already described. Penard ('02) mentions the discovery of a similar difference between the multinucleate specimens he collected in Switzerland and characterizes the larger group as "très grande, à bouche très largement ouverte, et toujours pourvue de noyaux en nombre considérable" (p. 410). Apparently no Arcellas with more than two or three nuclei have hitherto been reported from America.

Measurements were made of the diameter of the shell and diameter of the mouth of a sufficient number of specimens be-

Fig. 38 Arcella polypora. Family ap. 34. Pedigree showing two branch lines containing members with seven nuclei each. The members of the upper branch are larger than those of the lower branch.

longing to the groups with the large and small mouth openings to give satisfactory means. The data obtained are given in tables 22, 23, 24, 25, and 26. These data show that in every case the correlation between diameter of shell and diameter of mouth is high. The mean diameters and percentage of the diameter of the shell occupied by the mouth are given in table 22. In tables 22 and 26 are also presented measurements of the diameter of the shell and of the mouth of sixty large specimens collected from vegetation in the Wallkill River near Bloomington, New York, on September 4, 1918. The nuclear condition of these specimens could not be determined on account of the dense color of the shells, and they may have belonged to the binucleate species

TABLE 22

Arcella polypora. Table showing the relation between the diameter of the shell and the diameter of the mouth in five groups. The unit of measurement is 4.3 μ

GROUP	NUMBER OF SPECIMENS	MEAN DIAMETER OF SHELL	MEAN DIAMETER OF MOUTH	PERCENTAGE OF DIAMETER OCCUPIED BY MOUTH
Family ap. 5	26	28.15	11.85	42.10
Family ap. 34	43	24.23	9.91	40.90
Family ap. 69	4	34.50	14.00	40.53
Large specimens from Cold Spring Harbor	80	46.90	25.16	53.65
Large specimens from Bloomington, New York	60	62.48	30.28	48.46

TABLE 23

Arcella polypora. Family ap. 34. Correlation table for diameter of mouth and diameter of shell in forty-three specimens. The unit of measurement is 4.3 μ. Coefficient of correlation, 0.861 ± 0.028

DIAMETER OF SHELL	DIAMETER OF MOUTH					
	8	9	10	11	12	
21	1	2				3
22		5	1			6
23		3	1			4
24		2	7			9
25			10	2		12
26			2	4		6
27				2		2
28						
29					1	1
Totals	1	12	21	8	1	43

TABLE 24

Arcella polypora. Family ap. 5. Correlation table for diameter of mouth and diameter of shell in twenty-six specimens. The unit of measurement is 4.3 μ. Coefficient of correlation 0.735 ± 0.061

DIAMETER OF SHELL	DIAMETER OF MOUTH				
	10	11	12	13	
26	1	1			2
27		2	2		4
28		3	8		11
29			4	2	6
30			1	2	3
Totals	1	6	15	4	26

TABLE 25

Arcella polypora. Correlation table for diameter of mouth and diameter of shell in eighty large wild specimens collected at Cold Spring Harbor, Long Island. The unit of measurements is 4.3 μ. Coefficient of correlation, 0.719 ± 0.036

DIAMETER OF SHELL	DIAMETER OF MOUTH											
	20	21	22	23	24	25	26	27	28	29	30	31	
41	1		1										2
42	1	1	1						1				4
43			1	2									3
44				2	2								4
45			2	3	4	1							10
46				3	4	1	3	1	1				13
47				2	4	1	2		1				10
48					1	4	1	1	4			1	12
49						3	1	1	2				7
50					2	2	1		5	1			11
51								1					1
52										1			1
53											1		1
54												1	1
Totals...........	2	1	5	12	17	12	8	4	14	2	1	2	80

A. discoides. They are included here, however, because of the probability that they were multinucleate. The diameter of the shell ranged from 215 μ to 327 μ, and that of the mouth opening from 99 μ to 159 μ (fig. 39). The largest specimens of Arcella that have hitherto been recorded were 300 μ or less in diameter; these, therefore, are the largest that have ever been reported.

The differences between the percentages of the diameter occupied by the mouth in the different groups of specimens included in table 22 are of particular interest. If these groups all belong to the species polypora, then it seems evident that within the species there are lines that differ in the relative diameter of shell and mouth. Families ap. 5, ap. 34, and ap. 69 all fall in one group, with a mean percentage of about 41; the large specimens from Cold Spring Harbor in another, with a mean percentage of about 54, and the large specimens from Bloomington, New York, in a third, with a mean percentage of about 48. It is possible that the diameter of the shell and the diameter of the mouth are

TABLE 26

Arcella polypora. Correlation table for diameter of mouth and diameter of shell in sixty large specimens collected at Bloomington, New York, on September 4, 1918. The unit of measurement is 4.3 μ. Coefficient of correlation, 0.940 ± 0.010

DIAMETER OF SHELL	DIAMETER OF MOUTH															
	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	
50	1															1
51																
52		1		1												2
53																
54				1												1
55				1	1											2
56			1	1												2
57						1										1
58						1										1
59						1	1									2
60							3	2								5
61							1	4								5
62							1	3	2							6
63							1	3	1	2						7
64								1	1	2						4
65								1		5						6
66									2	2	2	1				7
67										1	1					2
68											2	1				3
69																
70										1						1
71																
72																
73																
74																
75																
76													1		1	2
Totals..	1	1	1	4	1	3	7	14	6	13	5	2	1		1	60

inherited independently, but it is clear from the tables that the correlation between these characteristics is very close both within families and in wild populations.

h. Summary of results of observations and experiments on Arcella polypora

1. The nuclear number of fifty-four wild specimens ranged from 3 to 10 and the diameters of the same from 25 to 33 units of 4.3 μ each. The coefficient of correlation between the nuclear number and the diameter of these specimens was $.156 \pm .089$.

2. Variations in diameter within families reared from wild specimens were much greater than those exhibited by Arcella dentata. This is due to changes in nuclear number within the families, such changes being accompanied by corresponding changes in diameter.

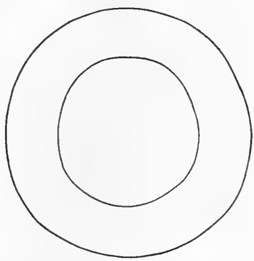

Fig. 39 Arcella polypora. Outline of a specimen from Bloomington, New York, with comparatively large mouth. × 207.

3. Different families differ constitutionally in diameter, and the data derived from the study of twenty-six families indicate that an indefinite number of families of different mean diameters exist in nature.

4. The correlation between parents and offspring with respect to diameter in a population consisting of twenty-five small families is very close ($.740 \pm .018$).

5. Breeding experiments with a large family (ap. 5) gave the following results:

a. Correlation between the diameters of parents and offspring within the family was marked ($.442 \pm .041$).

b. Selection within the family for specimens large and small in diameter was immediately effective and two lines were obtained showing a mean difference in diameter of 4.75 units of 4.3 μ each.

c. Changes in nuclear number occurred before and during the selection period, and all of the members of the low line were found to possess 4 nuclei, whereas those of the high line contained 5 or 6 nuclei.

d. Selection of the largest and smallest specimens within groups of members containing certain numbers of nuclei indicate the range of the diameters of specimens with each nuclear number. The correlation between nuclear number and diameter is very marked (.752 $\pm$ .018) and the mean diameter increases as the number of nuclei increases.

e. Changes in nuclear number occurred at irregular intervals and consisted usually of an increase or decrease of one nucleus. In several cases the number of nuclei doubled from 3 to 6. Changes in nuclear number were accompanied by changes in diameter, and in every case an increase in the number of nuclei led to an increase in diameter, and a decrease to a decrease in diameter.

f. The range in nuclear number was from 3 to 7, but specimens with 1 and 2 nuclei were obtained by bisecting specimens with 3 or 4 nuclei. Usually the parental condition was soon regained by the descendants of these pieces, but enough uninucleate and binucleate progeny were reared to prove that in these specimens, as well as in those with a greater number of nuclei, the diameter is closely correlated with nuclear number. No empty shells were formed at the time of a numerical increase in the nuclei.

6. Comparisons of the nuclear number and diameter of specimens in different families resulted as follows:

a. Among twenty-five families there were some whose members possessed few nuclei but were of large diameter and others whose members possessed a larger number of nuclei but were smaller in diameter.

b. Three small families (ap. 38, ap. 39, and ap. 69) were reared which exhibited to a marked degree this difference in diameter and nuclear number between families. Family ap. 38, whose members possessed 8 nuclei, had a mean diameter of 27.26 units; family ap. 39, whose members possessed 7 nuclei, had a mean diameter of 25.91 units, and family ap. 69, whose members possessed 6 nuclei, had a mean diameter of 34.22 units. These data should be compared with those obtained from family ap. 5, in which the specimens that possessed 7 nuclei had a mean diameter of 30.22 units.

c. Extended observations were made on family ap. 34. Here, as in families ap. 38 and ap. 39, the diameter associated with a particular number of nuclei was less than that in family ap. 5, and the number of nuclei ranged from 5 to 10, whereas in family ap. 5 the range (except when offspring were obtained from pieces) was from 3 to 7. Two branches of famly 34 whose members possessed 7 nuclei differed in mean diameter by 3.28 units; this indicates that lines permanently diverse in diameter but with the same number of nuclei might easily be isolated within this family.

7. A comparison of the relations between the diameter of the shell and the diameter of the mouth opening within different families and in wild populations indicates that groups of multinucleate specimens exist in nature that differ in the percentage of the diameter of the shell occupied by the mouth.

4. OBSERVATIONS AND EXPERIMENTS ON ARCELLA DISCOIDES

a. Pure lines

Many specimens of Arcellas that corresponded to the descriptions given by Leidy and others of A. discoides (fig. 40) were found in the same habitats where the multinucleate Arcellas were obtained. These were of many different sizes, but all resembled one another in possessing only 2 nuclei. Fifteen wild specimens of various sizes were chosen from a large number collected on duckweed and 7 families were reared from them. The distribution of the diameters of these families is given in table

27. Two groups may be recognized in the table, one with a distribution of from 21 to 26 units, and a mean of 23.32 units, and the other with a distribution of from 28 to 34 units, and a mean of 29.69 units. Families with diameters intermediate between these groups could no doubt have been reared and also families with smaller diameters, but the latter were too small to care for easily, and intermediate families were not reared because of lack of time. Variations in diameter in A. discoides are evidently less than in A. polypora and more nearly like those previously

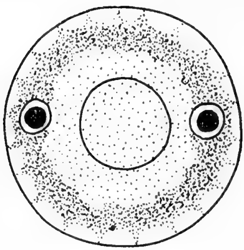

Figure 40

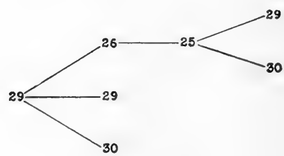

Figure 41

Fig. 40 Arcella discoides. Sketch showing the structure of a typical specimen. × 310.

Fig. 41 Arcella discoides. Family ad. 3. Part of pedigree showing the sudden appearance of a large temporary variation.

found in A. dentata. The smaller range of variation in A. discoides and A. dentata is probably due to the constancy in nuclear number, there being 2 nuclei in every specimen, except in a few unusual cases, whereas, as has been shown above, the large variations of A. polypora are accompanied by changes in nuclear number.

b. Variations in diameter and in nuclear number

Several abrupt but temporary variations were noted in the families of A. discoides. In family ad. 3 a parent 32 units in diameter and with 2 nuclei gave rise to an offspring 30 units in diameter, but with only 1 nucleus. The parent retained 3 nuclei, but the next offspring, which was the same size as the parent, possessed 2 nuclei and the parent also became binucleate.

The uninucleate offspring was provided with only a small amount of cytoplasm and died after a few days. In this case, evidently, something interfered with normal fission and the nuclei were unequally distributed, three remaining within the parent and only one being contributed to the offspring. The latter died, probably because of the small amount of cytoplasm it possessed. The parent with 3 nuclei may then have become binucleate again during the next fission, if only one of the nuclei divided and the four thus obtained were equally distributed between it and its next offspring.

TABLE 27

Arcella discoides. Table giving the distribution of diameters in seven families. The unit of measurement is 4.3 μ

NUMBER OF FAMILY	DIAMETER OF SHELL														NUMBER OF SPECIMENS	MEAN DIAMETER
	21	22	23	24	25	26	27	28	29	30	31	32	33	34		
1								1	3	5	4	1	2		16	30.45
2		11	22	6	4										43	23.07
3									13	21	23	11	5	1	74	30.69
5								1	2	3		1		2	9	26.89
8	2		5	2											9	22.78
10						1		1	1	3	7	4	2		19	30.74
13		2	1	3		3									9	24.11

Another case worthy of mention is that of the appearance of a large but temporary variation. This also occurred in family 3. The pedigree (fig. 41) shows how a sudden large decrease in diameter from 29 to 26 and then to 25 units was followed by a rapid increase from 25 to 29 and then to 30 units, which was near the normal family dimensions.

A third irregularity was noted in family ad. 10. A diameter much less than normal of several nearly related specimens was due to a crease in the shell which was passed on for one generation, and then disappeared, the full size of the specimens being regained. All of these specimens possessed the normal number of nuclei—2. The following series of figures gives the diameters in units of the immediate offspring of five successive generations, and the letter c indicates which specimens were creased.

32 – 29 – 28c – 26c – 30 – 31

The three cases just described indicate that within these families of A. discoides derived from one wild specimen by fission, there is a rather definite 'normal' condition as regards nuclear number, shell shape, and shell size, which may be disturbed temporarily, but is very soon regained.

c. The reproduction of uninucleate pieces

The data obtained from a cutting experiment make these results even more conclusive. A specimen from family ad. 3 was cut into two approximately equal pieces, each of which contained one of the two nuclei. The pedigree of these two pieces

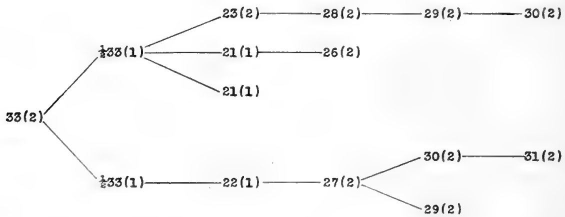

Fig. 42 Arcella discoides. Family ad. 3. Pedigree showing diameter and nuclear numbers of specimens derived from the two halves of a bisected specimen.

is given in figure 42, in which the large numbers indicate the diameter in units and the small numbers the number of nuclei. The first offspring of one piece was supplied with 2 nuclei, but the parental piece retained only 1 nucleus, and the two succeeding progeny had only 1 nucleus each. Evidently the nucleus of the parental piece divided into two and then one of these divided again before the first offspring was produced. Then when the first fission occurred two of the three nuclei thus formed were contributed to the offspring. The third generation exhibited an increase in size to near the mean diameter of the family, which was 30.69 units. In the history of the other piece nuclear doubling took place in the second generation and was accompanied by a sudden increase of 5 units. The mean diameter of the family was reached in the next generation. No empty shells were

formed at the time of nuclear doubling, as was found to be the case in A. dentata. The difference in size between the uninucleate and binucleate specimens of A. discoides is, however, very similar to that recorded above for A. dentata.

d. Summary of results of observations and experiments on Arcella discoides

1. Families reared from wild specimens of various sizes prove the existence of different pure lines with regard to diameter.
2. Variations in diameter within a family are less than those in A. polypora and more nearly like those in A. dentata. This is due to the constancy of the nuclear number.

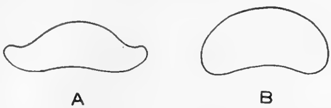

Fig. 43 Arcella vulgaris. Sketches showing side views of specimens belonging to two different families that differed heritably in the shape of the shell. *A* is from family ab. 7, and *B* from family avd. 1. × 207.

3. Uninucleate pieces of bisected specimens gave rise to a number of small uninucleate descendants, but nuclear doubling occurred in every case, without the intervention of an empty shell, and the mean diameter of the race was regained by members of the second generation.
4. The difference in diameter between the specimens with 1 nucleus and those with 2 nuclei is similar to that recorded for A. dentata and indicates that there is in this species a definite mass relation between nucleus and cytoplasm similar to that described in the latter.

5. OBSERVATIONS AND EXPERIMENTS ON ARCELLA VULGARIS

Many specimens of A. vulgaris were found clinging to the duckweed in the fresh-water ponds near Cold Spring Harbor. Eight specimens shaped like that shown in figure 43, *A*, were

isolated and families were reared from numbers 7 and 8. Family av. 7 consisted of twenty-eight specimens; among these were seven that possessed 3 nuclei. There were sixteen specimens in family av. 8, all of which were binucleate. In table 28 are given the distribution of diameters and the mean diameters of the binucleate specimens in family av. 7, the tri-nucleate specimens in family av. 7, and the specimens in family av. 8.

Another family, labeled avd. 1, was reared from a single specimen that possessed a shell like that shown in figure 43, *B*. There were thirty specimens in this family. The original progenitor was 31 units in diameter and its descendants ranged from 23 to 29 units, with a mean diameter of 25.47. The shape of the shell

TABLE 28

Arcella vulgaris. Table showing the distribution of diameter of the shell in binucleate and trinucleate specimens belonging to family av. 7, and in specimens belonging to families av. 8 and avd. 1. The unit of measurement is 4.3 μ

NUMBER OF FAMILY	DIAMETER OF SHELL											NUMBER OF SPECIMENS	MEAN DIAMETER
	23	24	25	26	27	28	29	30	31	32	33		
Av. 7: 2 nuclei.........			2	2		2	8	3	3		1	21	28.86
Av. 7: 3 nuclei........						1	1	2	3			7	30.00
Av. 8: 2 nuclei........				1		8	2	4	1			16	28.69
Avd. 1: 2 nuclei.......	4	10	2	5	4	3	1		1			30	25.47

was constant within this family as well as within families av. 7 and av. 8 described above, hence we may conclude that shape as well as size is a character that is inherited within families of Arcella vulgaris.

The results of these studies on Arcella vulgaris may be stated as follows:

1. Families reared in the laboratory from wild specimens reveal the existence of pure lines in this species as regards diameter.

2. Variations in diameter within families are similar to those observed in A. dentata and A. discoides.

3. In one family a series of specimens appeared that possessed 3 nuclei. These were all larger than the specimens with 2 nuclei, and indicate that here, as in the other species described, there is

a correlation between size and nuclear number and that the greater the number of nuclei, the larger the size.

4. Shape as well as size is a character that is inherited within families of Arcella vulgaris.

6. THE RELATIONS BETWEEN CHROMATIN MASS AND CELL SIZE

What the essential differences are between families ap. 5 and ap. 34 of Arcella polypora is a problem that involves the study of the relation between chromatin mass and cell size, since the suggestion presents itself at once that there may be a quantitative difference in the amount of chromatin in the nuclei possessed by members of the two families, but that the chromatin is qualitatively approximately alike. Before considering these two families of A. polypora it will be instructive to examine the conditions within two families of A. dentata.

a. Chromatin mass and cell size within two families of Arcella dentata

These two families had the following history before they were used for the studies reported below. Line 150.2ba, which was part of family 150, is fully described in the first part of this contribution, having been used in the cutting experiments. The original progenitor was collected at Baltimore on December 27, 1917, and when the experimental work upon its offspring was discontinued a few specimens were kept in a Syracuse watch-glass so that the line would not die out. These were examined every day or two to see if any conjugation was taking place, and about once each week six of the youngest specimens were transferred to another watch-glass containing fresh food. No conjugation was observed during the period when they were thus being continued in mass culture. During the winter and spring months this line produced a generation every two and one-half days and there is no reason to suppose that this rate of reproduction changed after the mass-culture method was begun, hence at the time the chromatin mass studies were begun the specimens used represented about the 93rd generation. During the

pedigree work carried on with this line, all of the specimens examined possessed 2 nuclei and the mean spine number was 14.25, and the mean diameter was 33.16 units. A distinct change took place during the period of mass culture since the mean spine number of the forty specimens used for chromatin-mass measurements had decreased to 13.32 and the mean diameter of the same specimens had likewise decreased to 31.43 units. Whether this decrease was due to changes in the food conditions or to temperature or to some other environmental factor is not known. The rate of reproduction, on the other hand, increased from one generation in two and one-half days to one generation in slightly less than two days. This was probably due to the higher temperature of summer.

Line 58 e.g. was a continuation of the high branch of the low line of family 58 described in a previous report (Hegner, '19). It was kept in mass culture under conditions similar to those described for family 150.2ba. The specimens used for chromatin-mass measurements represented about generations 129 to 136. When the pedigree culture work was discontinued the mean spine number was 11.38 and the mean diameter was 27.22 units. The mean spine number of the forty specimens whose chromatin mass was measured was 10.29 and the mean diameter was 24.45 units. Here, as in family 150.2ba, there is a similar decrease in both spine number and diameter, and since both families agree in this respect it seems probable that the environment is responsible for the change. It should be noted that during the selection work already reported simultaneous changes in spine number and diameter took place on several occasions in all the lines at the same time, probably because of some change in the environment.

The complete history of A. dentata from one generation to the next is not yet fully known, but judging from the small number of specimens that are found in division during the period of daylight, fission probably occurs some time during the night. Popoff ('08) has found that in ciliates there is a gradual growth of nucleus and cytoplasm during the first part of the period between successive fissions and then a very rapid nuclear enlargement

just before division occurs. Conditions may be somewhat similar in Arcella, and accordingly measurements were made of specimens of both lines at various times during the day, so that an average size might be obtained.

One very great advantage possessed by Arcella over the other protozoa thus far studied with a view to the solution of nucleo cytoplasmic problems is the fact that its nuclei are of the supposedly primitive type, having all their chromatin clumped to-

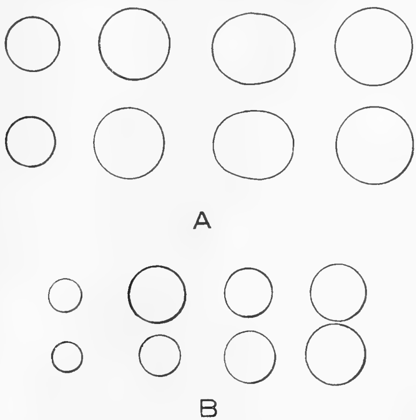

Fig. 44 Arcella dentata. Outlines of the chromatin masses within the nuclei of living specimens. In *A* the pairs represent the chromatin masses from single specimens from the larger line (150.2ba); these were selected to show the range of variation. In *B* are similar pairs from the smaller line (58eg.). × 900.

gether in a compact mass in the center. It is thus easy to measure the chromatin mass separated out from the other nuclear constituents. This chromatin mass is spherical or nearly so, and is distinctly visible in the living animal. Camera-lucida drawings of the nuclei of living specimens were made; a no. 10 ocular and a 3-mm. water-immersion lens were used for this purpose. This combination was found to give a magnification of 900 diameters. A series of these drawings is reproduced in figure 44, so as to show the various sizes of the chromatin masses within

the two lines studied, and also to indicate the differences in diameter of the chromatin masses between the two lines.

The diameters of the drawings were measured in units of 0.25 mm. Table 29 gives the distribution of the diameters thus obtained. The range of variations was in line 150.2ba. from 36 to 56 units, with a mean diameter of 47.82 units; and that in line 58eg. was from 24 to 41 units with a mean diameter of 35.66 units. There was thus a difference of 12.16 units between the mean diameters of the chromatin masses in the two lines. These data prove conclusively that the diameters of the chromatin masses within these two lines were different and that the chromatin

TABLE 29

Arcella dentata. Table showing the distribution of the diameters of the two chromatin masses in forty specimens each of lines 150.2ba and 58eg. The chromatin masses within the living animals were drawn with a camera lucida magnified 900 diameters, and the diameters of these drawings were there recorded in units of 0.25 mm.

LIME	DIAMETERS IN UNITS OF 0.25 MM.																																MEAN DIAMETER
	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	
150.2 ba.													1	1	1	2	1	1	4	3	1	3	7	4	10	7	15	3	9	4	2		1	47.82
58 eg..	1	1		1	5	3	1		2	1	8	3	17	8	10	7	7	5																35.66

masses in the smaller line were much smaller than those in the larger line.

Another fact brought out by these measurements is that the two chromatin masses in any specimen are often exactly equal in size and usually almost of the same size. The differences in diameter between members of the pairs of chromatin masses in the specimens are tabulated in table 30. In line 150.2ba. the members of twelve pairs were equal in size and those of the other twenty-eight pairs differed from each other in diameter from 1 to 4 units, the average difference for the forty pairs being 1.5 units. In line 58eg. the members of seventeen pairs were equal in size and those of the other twenty-three pairs also differed from each other from 1 to 4 units in diameter, the mean difference being 0.98 unit.

TABLE 30

Table showing the differences in diameter between the chromatin masses of the two members of the pairs of nuclei in specimens from families 150.2b and 58eg. of Arcella dentata and families ap. 5 and ap. 34, of A. polypora. The nuclei of the living specimens were drawn at a magnification of 900 diameters and then their diameters were measured in units of 0.25 mm.

FAMILY	DIFFERENCE IN PAIRS IN UNITS OF 0.25 MM.						
	0	1	2	3	4	5	Mean difference between members of pairs
150.2ba	12	9	9	7	3		1.50
58eg	17	11	9	2	1		.98
Ap.5	14	26	22	12	1		1.47
Ap.34	22	26	18	5	3	1	1.26

b. Chromatin mass and cell size within two families of Arcella polypora

The chromatin-mass condition within families ap. 5 and ap. 34 of Arcella polypora was more difficult to determine because of the larger number of nuclei and their smaller size. However, satisfactory camera drawings were made of the chromatin masses of twenty-five specimens in each family. The number of chromatin masses in a specimen ranged from 5 to 7 in family ap. 5 and from 4 to 10 in family ap. 34. Only the drawings of three chromatin masses of each specimen were measured, thus giving seventy-five measurements for each family. Table 31 shows that

TABLE 31

Arcella polypora. Table showing the distribution of the diameters of three of the chromatin masses in twenty-five specimens each of families ap. 5 and ap. 34. The chromatin masses within the living animals were drawn with a camera lucida magnified 900 diameters, and the diameters of these drawings were then recorded in units of 0.25 mm.

FAMILY	DIAMETERS IN UNITS OF 0.25 MM.																
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Mean diameter
ap. 5						2	3	5	11	14	11	8	11	6	2	2	25.91
ap. 34	2	1	7	5	5	8	14	12	10	6	4			1			21.93
Difference																	3.98

in family ap. 5 the chromatin masses ranged in diameter from 21 to 31 units, with a mean diameter of 25.91 units; whereas in family ap. 34 they ranged from 16 to 29 units in diameter, with a mean diameter of 21.93 units. The difference between the mean diameters of the chromatin masses in the two families is therefore 3.98 units.

With these data it was possible to determine whether there is a similar relation between the quantity of chromatin and the quantity of cytoplasm within the two families. The actual mean

TABLE 32

Arcella polypora. Table showing the relation between the diameter of the shell and the volume of the chromatin mass in specimens belonging to families ap. 5 and ap. 34

	NUMBER OF NUCLEI							
	3	4	5	6	7	8	9	10
Mean diameter of a shell in microns. Family ap. 5	110.08	112.36	117.65	127.67	129.95			
Volume of chromatin in cubic microns. Family ap. 5	586.32	781.76	977.20	1172.64	1368.08			
Mean diameter of shell in microns. Family ap. 34			105.22	106.08	112.02	115.67	120.27	123.45
Volume of chromatin in cubic microns. Family ap. 34			594.20	713.04	831.88	950.72	1069.56	1188.40

volume of the chromatin masses in the two families was first ascertained. This proved to be 195.44 cubic microns in family ap. 5, and 118.84 cubic microns in family ap. 34. With these data table 32 was constructed; this table shows the mean diameter of the shell in microns in families ap. 5 and ap. 34 with respect to the number of nuclei, and the mean volume in cubic microns of the chromatin masses in each group. For example, the table shows that the mean diameter of the shells of the members of family ap. 5 that possessed 3 nuclei was 110.08 μ and that the mean volume of the three chromatin masses was 586.32 cubic

microns. The following data selected from this table show that in members of the two families with shells nearly alike in diameter the nuclear number is different, but the volume of the chromatin is very nearly the same.

FAMILY	NUMBER OF NUCLEI	MEAN DIAMETER OF SHELL IN MICRONS	MEAN VOLUME OF CHROMATIN IN CUBIC MICRONS
ap. 5	4	112.36	781.76
ap. 34	7	112.02	831.88
ap. 5	5	117.65	977.20
ap. 34	8	115.67	950.72
ap. 5	6	127.67	1172.64
ap. 34	10	123.45	1188.40

The differences in chromatin volume between the pairs of groups are not greater than would be expected as a result of slight errors in the measurements. In figure 45, *A*, are shown outlines of the eight chromatin masses from a typical specimen from family ap. 34, and in figure 45, *B*, outlines of the five chromatin masses from a typical specimen of the same size from family ap. 5.

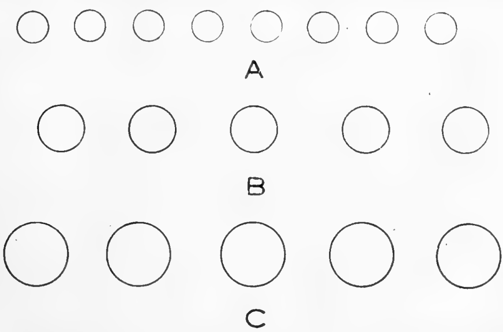

Fig. 45 Arcella polypora. Outlines of the chromatin masses within the nuclei of living specimens. *A* = eight chromatin masses from a typical specimen from family ap. 34. *B* = five chromatin masses from a typical specimen of the same size from family ap. 5. *C* = five chromatin masses from a very large specimen, ap. 74. × 900.

The volumes of the two groups of chromatin masses are approximately the same.

A large amount of time and effort was devoted to an attempt to rear young from the very large specimens of A. polypora collected at Cold Spring Harbor (p. 57, table 25), but although every care was taken only a few offspring were obtained. The nuclei of these were not measured at the time, but later a wild specimen was collected in which the nuclei could be seen very clearly. There were 5 nuclei in this specimen; these had a mean diameter of chromatin mass of 11.56 μ, and a mean volume of chromatin mass of 808.86 cubic microns. The shell measured 202 μ in diameter. When these measurements are contrasted with those given above for families ap. 5 and ap. 34, and when figures 42, A, B, and C. are compared, it becomes evident that the relation between the quantity of chromatin and the quantity of cytoplasm is here similar to the relations established for families ap. 5 and ap. 34.

c. The possible relation between the chromatin mass and the death of certain specimens of Arcella dentata

Among the lines obtained in family 58 of A. dentata during selection experiments (Hegner, '19) was one labeled 58ed. The members of this line were heritably different in size from the other lines studied, being much smaller than any of the rest and possessing fewer spines. The line 58e. from which it was derived, had a mean diameter of 27.05 units of 4.3 μ each and a spine number of 10.99, whereas line 58ed. had a mean diameter of only 23.51 units and a mean spine number of 9.91. From this small line 58ed. there was obtained a line 58edb. containing extremely large specimens. These had a mean diameter of 35.54 units and a mean spine number of 17.54. However, in spite of the greatest care, only a few of these large specimens were reared, and these all died in the course of several weeks.

Drawings of one chromatin mass in each of four specimens belonging to this large line were made, and similar drawings of chromatin masses in four specimens of the small parent line (fig.

46). These drawings were then measured; the results are presented in table 33. Although the specimens in line 58edb. are very much larger than those in line 58ed., the mean diameter of the chromatin mass within their nuclei is almost exactly the same, being 8.73 μ in the former and 8.44 μ in the latter. These data suggest that the large specimens were unable to exist because of the small quantity of chromatin they possessed in comparison with the large amount of cytoplasm. Why the chromatin did not increase in amount to keep pace with the enlargement of the cytoplasmic body is a question that cannot now be answered.

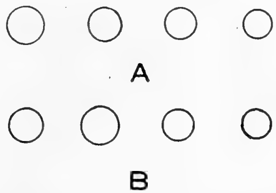

Fig. 46 Arcella dentata. Outlines of the chromatin masses within the nuclei of living specimens. *A* = one chromatin mass from each of four specimens from the small parent line, ed. *B* = one chromatin mass from each of four specimens from the large branch line, edb. × 630.

TABLE 33

Arcella dentata. Table showing the relation between the diameter of the shell and the number of spines, and the diameter of the chromatin mass in four specimens each in lines 58 edb. and 58 edc.

LINE 58 edb.			LINE 58 edc.		
Diameter of shell in microns	Number of spines	Diameter of chromatin mass in microns	Diameter of shell in microns	Number of spines	Diameter of chromatin mass in microns
159	20	8.73	103	10	9.52
172	20	9.92	95	8	8.73
151	19	8.73	95	10	7.94
142	16	7.54	99	10	7.54
Means 156	18.75	8.73	98	9.50	8.44

d. Summary of the studies of chromatin mass and cell size

1. Measurements were made of the chromatin masses contained in the nuclei of eighty specimens of A. dentata, forty specimens belonging to family 150 and forty specimens belonging to family 58. The two chromatin masses in any one specimen were very nearly equal. The chromatin masses in the different specimens varied considerably in size, but the mean quantity of chromatin in the specimens of family 150, which were large, was much greater than the mean quantity of chromatin in the smaller specimens belonging to family 58.

2. Similar measurements were made of the chromatin masses contained in the nuclei of members of families ap. 5 and ap. 34 of A. polypora. It was found that in specimens of family ap. 5, which were large in comparison with the number of nuclei, the chromatin masses were large, whereas in specimens of family ap. 34, which were smaller in comparison with the number of nuclei, the chromatin masses were smaller. The quantity of chromatin, however, contained in specimens of the same size within the two families was very nearly the same. In these specimens, as in those of A. dentata, although there was considerable variation in the chromatin masses within different specimens, the chromatin masses within any one specimen were approximately the same.

3. Measurements of the chromatin masses in specimens of two diverse lines of A. dentata indicate that the specimens in the larger line all died because of the small amount of chromatin they possessed in comparison with the size of the cytoplasmic body.

7. DISCUSSION

Certain topics that have been mentioned in the foregoing pages are worthy of further elaboration and are discussed in the following paragraphs.

1. The nucleocytoplasmic-relation theory

This theory is based on the idea that the nucleus is the dynamic center of the cell. The nuclear membrane is considered as

semipermeable, allowing a constant, but selected flow of materials from the nucleus into the cytoplasm and vice versa. Involved in the theory is also the idea expressed by Sachs in 1892, and the following year by Strassburger in a slightly different form. According to Sachs, the nucleus and that part of the cytoplasm that falls within its 'sphere of influence' make a morphological and physiological unit. He proposed the term 'energid' for such a unit, and this term might well be employed in describing conditions in Arcella, since, as we have seen, the nuclei in both the binucleate and multinucleate species are approximately equidistant from one another, as though each were acting as the dynamic center of an equal portion of the cytoplasm. Strasburger ('93) recognized a limit to the distance through which the metabolic interchange between nucleus and cytoplasm could take place, and was able to show that in the cells of certain plants the ratio between nuclear size and cell size was fairly constant. He accounted for cell division on the hypothesis that when the cytoplasm had increased so as to extend beyond the working sphere of the nucleus the normal ratio was regained by the division of both nucleus and cytoplasm into two. The nucleocytoplasmic-relation theory as we now know it is largely due to the investigations carried on by Richard Hertwig and his students. Hertwig ('03) came to the conclusion that in every cell under normal conditions there is a definite relation between the quantity of cytoplasmic and nuclear materials, which he called the 'Kernplasmarelation.' In favor of this theory is the discovery by Gerassimoff ('02) that in Spirogyra if nuclei are segregated in one cell following cell division this binucleate cell becomes larger than the normal uninucleate cells; and the generalization of Boveri ('02, '05) arrived at from studies of the cells of sea-urchin larvae, that "Die Grösse der Larvenzellen ist eine Funktion der in ihnen enthaltenen Chromatinmenge, und zwar ist das Zellvolumen der Chromosomenzahl direkt proportional." According to Hertwig, the magnitude of the K/P (nucleus/cytoplasm) ratio determines vital processes such as growth, division, senescence, and physiological degeneration. The latter, for example, result

from an abnormal increase in the ratio (i.e., in the growth of the nucleus), and Rhizopoda and Infusoria in which this condition was induced by overfeeding either died or else regained the normal ratio by direct elimination of nuclear material or by conjugation. The intervals between successive divisions were divided by Hertwig into two periods: 1) a period of 'funktionelles Wachstum,' during which the cytoplasm grows more rapidly than the nucleus, leading to an abnormal K/P ratio, and, 2) a period of 'Theilungs-wachstum,' during which the normal K/P ratio (the 'Kernplasma-Norm') is regained by the rapid growth of the nucleus, and at the end of which cell division occurs. The end of the period of functional growth when the K/P ratio is abnormal is the moment of 'Kernplasma-Spannung.' Popoff ('08) believes he has proved by experiments on Frontonia and other ciliates that division and the plane of division are determined at the moment of nucleo-cytoplasmic tension. For example, if pieces of cytoplasm were removed during the period of functional growth, the cytoplasm continued to increase until the nucleocytoplasmic norm was established, but if they were cut away during the period of division-growth, division occurred without the regaining of the nucleocytoplasmic norm and the organism divided into unequal parts in the plane which was median before the operation.

Many external and internal conditions are supposed to affect the normal K/P ratio. Thus Hertwig found that the ratio was increased if the organism were subjected to low temperatures and was diminished at higher temperatures. For example, the average K/P ratio for Dileptus gigas at 8°C. was 1/7, and at 25°C. was 1/13. Similar results were obtained by Popoff ('08) on Stylonychia. This investigator used the ratio P/K (cytoplasm/nucleus) and found that the ratio decreased as the temperature decreased; e.g., it was 80.7 at 25°C., 77.4 at 17°–19°C., and 74 at 10°C. Rautmann's ('09) experiments on Paramecium also confirm Hertwig's conclusions. He found that at temperatures above 25°C. the K/P ratio decreased. The confirmation offered by Prandtl's ('06) studies on Didinium

and Dileptus is particularly striking. Not only was the K/P ratio large when the organisms were subjected to a low temperature, but when a higher temperature was subsequently substituted conjugation took place involving a reorganization of the nuclei and the acquisition of the normal K/P ratio for the higher temperature.

Other external factors that appear to affect the nucleocytoplasmic ratio are overfeeding and starvation. Hertwig caused an increased K/P ratio in Actinosphaerium and certain Infusoria by overfeeding, and Kasanzeff ('01) obtained a similar result in Paramecium by starving the organisms.

Many investigators have studied the nucleocytoplasmic relations in Protozoa and in Metazoa, confirming in part the conclusions reached by Hertwig and his followers. The work of Minot ('08), however, leads to a theory directly opposed to that of Hertwig. Minot finds that in segmenting eggs the amount of nuclear material increases as compared with the quantity of cytoplasm, and concludes that this increase is indicative of the process of rejuvenescence. Rejuvenescence is thus revealed as an increase of the nuclear material, and senescence as an increase and differentiation of the cytoplasm. When applied to Protozoa, Minot's theory requires "in those cells which are old an increase in the proportion and in the differentiation of the protoplasm [cytoplasm], and consequently a diminution in the relative amount of nucleus" ('08, p. 231).

Conklin ('12) disagrees with both Hertwig and Minot regarding the connection of the nucleocytoplasmic relation with senescence and rejuvenescence, but accounts for these phenomena by decreases and increases in metabolism. His studies of Crepidula show that great variation exists in the nucleocytoplasmic relations of different blastomeres, but that the ratio between nucleus and cytoplasm is similar in corresponding blastomeres of different eggs. He does not believe that cell division in Crepidula is initiated by limitations of the working sphere of the nucleus nor by a nucleocytoplasmic tension; but is related to the rate and nature of the metabolism in the cell. Somewhat similar conclusions were reached by Woodruff ('13)

from his investigations of Oxytricha fallax. In this ciliate "The size of the cell and the size of the nucleus as well as the nucleocytoplasmic relation are interpreted as an incidental result rather than a cause of the rate of cell division" (p. 22).

Only a few scattered studies of the chromatin-cytoplasm relation have been published, and these are for the most part unsatisfactory. Boveri ('02), as mentioned above, attributed cell size in larval sea-urchins to chromatin number; whereas, Erdmann ('09), who also studied sea-urchins' eggs, concludes that the chromatin mass rather than the number of chromosomes is the size-determining factor. In plants also the relation between chromatin mass, chromosomes, and cell size have been considered. Gates ('09), for example, believes that the cells of Oenothera gigas are large because of the double number of chromosomes present, and not merely because of an increase in chromatin mass.

The theory offered by Hertwig to account for the various complex processes that occur during the life cycles of the Protozoa appears at first plausible, but will not withstand close analysis. The data are entirely inadequate to sustain the claims of the theory, but investigations of the actual relations of nucleus, chromatin, and cytoplasm to one another and their relations to external heritable characteristics are very desirable, especially since thay may throw light upon the important genetic studies of the Protozoa that have been published, especially by Jennings and his students.

2. *Nucleocytoplasmic relations in Arcella*

All of the data gathered together in the course of the investigations herein described favor the hypothesis that within a certain line of a certain species of Arcella there is a definite quantitative relation between nucleus and cytoplasm. Of interest in this connection is the fact that in both binucleate and multinucleate specimens the nuclei, although free to move about within the cytoplasmic mass, become arranged in such a manner that they are equidistant from one another, and

hence have each an equal amount of cytoplasm with which to interact. This is very clearly indicated in figures 30 and 34, which show the positions of the nuclei in typical specimens of Arcella polypora. That the nuclei are free to move about to a certain extent is shown in figure 47, which gives the positions assumed by the two nuclei of a specimen of Arcella dentata at intervals of about twenty-four hours. It seems clear, therefore, that they are not held in place by a network of fibers or by other structures in the cytoplasm. Also, when nuclei are forced out of place by pressing upon the shell with a blunt needle, they become equidistant from one another again in a short

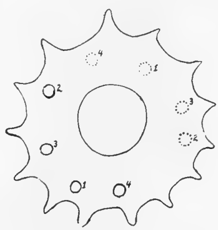

Fig. 47 Arcella dentata. Outlines of a specimen showing the positions of the two nuclei at intervals of twenty-four hours. One nucleus is represented by a solid circle, the other by a dotted circle. Their positions are numbered as follows: 1, March 14; 2, March 15; 3, March 16; 4, March 18. × 207.

time. The freedom of the nuclei from cytoplasmic attachment was strikingly exhibited during the microdissection experiments, since a slight pressure on the shell, after a break had been made near them (fig. 18), was sufficient to cause them to pop out through the shell opening, usually in a condition entirely free from any accompanying cytoplasm.

The principal data that have been presented in the preceding pages in favor of the presence of a definite quantitative relation between nucleus and cytoplasm may be summarized briefly as follows:

Arcella dentata. When part of the shell and a small portion of the cytoplasm were removed, but both nuclei were left intact,

the immediate offspring were smaller than the original parent, but their offspring regained the dimensions of their mutilated grandparent—dimensions 'normal' for the line to which they belonged. The size of the immediate offspring was less, probably because of the removal of part of the parent shell, thus limiting the capacity of the part of the shell that remained.

Uninucleate specimens descended from parts of binucleate specimens acquired within a few generations dimensions that varied within a narrow range and that were measurably smaller than the parent line of binucleates. At first the uninucleate descendants of small pieces were smaller than those of larger pieces, but the size which was later regained was the same in both cases, showing that the nuclei were alike and controlled the quantity of cytoplasm that accompanied them. Furthermore, the uninucleates of different lines showed a difference in their normal size corresponding to that of their progenitors. For example, in line 150 the mean diameter of the binucleates was about 34 units of 4.3 μ each and that of the uninucleates about 26.50 units, whereas, in line 58 the mean diameter of the binucleates was about 27 units and that of the uninucleates about 21 units. Furthermore, in each line the mass of cytoplasm contained within the uninucleates was approximately one-half of that within the binucleates.

The change from the binucleate to the uninucleate condition at the time of empty shell formation was always followed by a gradual increase in the dimensions of the offspring for the three or four succeeding generations, but when the normal size for the line was reached no further enlargement occurred. On the other hand, on the several occasions when, because of some unknown stimulus, binucleates became uninucleate, their uninucleate offspring were smaller, thus indicating that the decrease in size of the uninucleates throughout the experiment was not due to injuries caused by the operations, but to the fact that only one nucleus was present.

The persistence of the mass relations between nucleus and cytoplasm is especially well illustrated by the cutting experiments upon uninucleates. In each of five successive lines of

uninucleates, half of the shell with its contained cytoplasm was removed from certain specimens, yet in each case the uninucleate progeny regained after a few generations the normal size of the uninucleates of the line, and the binucleates later derived from these subsequently reached the dimensions of normal binucleates of the line.

Confirmative data were obtained from the experiments performed on the uninucleate half-specimen that appeared in the cultures, and also from the microdissection experiments during which one nucleus but no cytoplasm was removed. Most of the specimens from which the nucleus only was removed assumed the binucleate condition immediately, and as suggested on a preceding page, this was probably due to the fact that the single nucleus that remained was associated with a mass of cytoplasm that extended beyond its sphere of influence. The normal condition could be regained either by the acquisition of a second nucleus or by a decrease in the mass of the cytoplasm. The former process occurred in all cases.

Arcella polypora. The observations and experiment on Arcella polypora demonstrate that a constant mass relation exists between cytoplasm and nucleus in these organisms even more clearly than do those on A. dentata.

In family ap. 5 the coefficient of correlation between diameter and nuclear number was 0.752 ± 0.018 (table 15) even at a time when selection was being practiced for large and small diameters within the groups containing certain numbers of nuclei. Later during a non-selection period the correlation with respect to these characteristics within this family rose to 0.818 ± 0.023 (table 17). In family ap. 34 the coefficient of correlation between the diameter of the shell and nuclear number was 0.699 ± 0.020 (table 21). It is assumed that the diameter of the shell is a reliable index of the size (mass) of the organism, and hence it follows that in any particular family a large number of nuclei is accompanied by a greater mass of cytoplasm than a smaller number of nuclei. This relation between the number of nuclei and the cytoplasmic mass is also illustrated by the data collected at the time when changes in the number of

nuclei occurred. Thus, as shown in table 16 and on pages 44 and 45, offspring whose nuclear number is less than that of the parent are almost invariably smaller than the parent, and when nuclear doubling occurs, as, for example, when a parent with 3 nuclei gives rise to progeny with 6 nuclei, the immediate result is a more decided increase in the diameter of the latter.

Bisected specimens of A. polypora belonging to family ap. 5 behaved very much as did those of A. dentata except that the acquisition of new nuclei was not accompanied by empty shell formation. The results of the cutting operations confirm those obtained from the study of uninjured animals, since specimens with 1 or 2 nuclei were smaller than those with 2 or 3 and an increase in nuclear number was always accompanied by an increase in size. In A. polypora, as in A. dentata, there was a tendency to return, after the operations, to the condition 'normal' for the line, and it was possible to procure only a few specimens with 1 or 2 nuclei, since such organisms almost immediately acquired a number of nuclei near the average for the line from which they had been derived. Other families of A. polypora were similar to families ap. 5 and ap. 34 in their nucleocytoplasmic relations as described on pages 51 and 52.

Arcella discoides. In this species the variations in size were small as compared with those encountered in A. polypora and more nearly like those described for A. dentata. This is easily explained by the fact that the variation in nuclear number was never more than one. Uninucleates resulting from the bisection of a normal binucleate specimen reacted, as shown in figure 42, very much as did those of A. dentata, except for the absence of empty shells at the time of nuclear doubling.

Arcella vulgaris. In this species further evidence was obtained of a definite nucleocytoplasmic mass relation, since, as indicated in table 28, the binucleate specimens in family av. 7 had a mean diameter of 28.86 units of 4.3 μ each, whereas the trinucleate specimens had a mean diameter of 30 units.

3. Chromatin-cytoplasmic relations in Arcella

The morphology of the nuclei of Arcella makes it possible to study the mass relations between the chromatin and the cytoplasm, since the former occurs as a nearly spherical body suspended in the nuclear sap. This 'chromatin mass' may contain plastin or some other substance besides chromatin, but the amount of chromatin probably varies as the size of the mass. It was very easily determined in Arcella dentata that in two different lines that differed in size the chromatin masses within the nuclei of the larger were greater than those within the nuclei of the smaller. A clear relation between chromatin mass and cytoplasmic mass was thus established.

A similar conclusion was reached with respect to different lines of Arcella polypora. In this species there was found to be a constant difference in size between specimens with the same number of nuclei belonging to different lines. When, however, the masses of these nuclei were computed and the total volume of chromatin was determined, the results showed that here also there is a definite mass relation between chromatin and cytoplasm. The number of nuclei might be different in specimens belonging to different lines, but the size is the same whenever the total volume of chromatin is the same.

In one case the data seem to favor Minot's theory that senescence and death are due to an excess in the amount of cytoplasm. From a line of Arcella dentata containing very small specimens there was derived a line consisting of enormously large ones. These, after several generations, failed to reproduce, and on examination, their nuclei were found to be no larger than those of small members of the parent line. Their subsequent death may have been due to the failure of the organisms to regain the normal racial relations between chromatin and cytoplasm.

4. Changes in nuclear number and the formation of empty shells

This subject involves several interesting questions that cannot be answered with any degree of certainty. Some of these questions are as follows:

Why were new shells produced by apparently normal specimens in the various lines of Arcella dentata and A. polypora and then cast off empty? Why was the change from the uninucleate to the binucleate condition in A. dentata always accompanied by empty shell formation? Why were not changes from the binucleate condition to the uninucleate condition in A. dentata likewise accompanied by the throwing off of empty shells? Why were no empty shells formed in A. polypora and A. discoides when changes in nuclear number occurred? Why did the uninucleates in the lines of A. dentata and A. discoides all finally become binucleate? What determines the generation when nuclear changes occur? Why did uninucleate and binucleate specimens of A. polypora obtained from pieces all produce progeny with a greater number of nuclei, and thus return to the average condition of the line? Why did not the trinucleate race of A. vulgaris persist?

The formation of empty shells by apparently normal specimens of A. dentata may have resulted from the failure of the nuclei to divide. This may in turn have been due to the lack of sufficient cytoplasm to initiate division of the nuclei, although the factors that are responsible for cell division were in operation.

Similarly in A. polypora cell division may have begun at a time when the total amount of chromatin within the organism was in excess, but was not completed normally because of the presence of an insufficient amount of cytoplasm.

The production of an empty shell when nuclear doubling occurs in A. dentata may be accounted for in the following way. Having reached the stage when division normally takes place, the nucleus of the organism divides and a new shell is formed. Both nuclei and all of the cytoplasm remain in the parent shell, however, instead of one nucleus and half of the cytoplasm separating from the rest and occupying the new shell, as usual. What causes this process is not clear. In A. discoides and A. polypora under similar conditions no empty shells are formed. Racial differences must be assumed to account for this condition. Evidently cell division in these

two species is not begun until there is a sufficient number of nuclei in the parent to fully supply both parent and offspring.

Empty shells were not formed when changes from a greater to a lesser number of nuclei occurred, because there were nuclei enough for both parent and offspring, and hence division of the cytoplasm could proceed as usual.

Changes from the binucleate to the uninucleate condition in A. dentata were probably due to the failure of the nuclei to divide at the time of fission, hence both parent and offspring received but a simple nucleus.

The change from the binucleate to the trinucleate condition noted in A. vulgaris may have resulted from two successive divisions of one of the nuclei of the original parent before fission occurred, and the subsequent distribution of three nuclei each to both parent and offspring.

The change from a lesser to a greater number of nuclei probably depends upon several factors. The results of the microdissection experiments indicate that nuclear doubling in A. dentata occurs more quickly when a larger mass of cytoplasm is present than normally comes under the sphere of influence of one nucleus. The amount of cytoplasm is in turn determined by the capacity of the shell. It has been shown that variations in the dimensions of the new shell are never very great, apparently being limited by the size of the parent shell, and that a large internal change, such as nuclear doubling, is not immediately followed by a correspondingly large variation in the progeny, but requires several generations for its complete realization so far as external characteristics are concerned. It appears from these data that the uninucleate specimens remained uninucleate until a variation in the dimensions of the shell occurred which increased the shell capacity sufficiently to allow an increase in the cytoplasm beyond the amount usually associated with a single nucleus. The presence of cytoplasm outside of the 'sphere of influence' of a single nucleus may be the stimulus that initiates the process of nuclear doubling. Thus the generation in which this process occurs is not definitely fixed, but depends upon variations in the shell capacity of the organism.

Variations observed in the number of nuclei in A. dentata, A. discoides, and A. vulgaris were slight; in A. vulgaris specimens were obtained which possessed 1, 2, and 3 nuclei, respectively, whereas in A. dentata and A. discoides all of the specimens were either uninucleate or binucleate. For these three species the binucleate condition is evidently normal and all of the uninucleates and trinucleates eventually reverted to this state. In A. polypora, however, considerable variation in nuclear number was noted within each pure line. Thus the number in family ap. 5 ranged from 3 to 7 and in family ap. 34 from 5 to 10. Nuclear changes were of frequent occurrence in these families and reversion to the modal condition was constantly taking place.

5. Pure lines

The data already published (Hegner, '19) prove that a great many pure lines exist in Arcella dentata with respect to spine number and diameter of the shell. They also demonstrate a close correlation between these two characters. The observations recorded in the present contribution indicate that pure lines likewise exist in the other species of Arcella that have been studied. The characteristics that have been noted particularly are: 1) in Arcella vulgaris, the diameter and shape of the shell; 2) in A. discoides, the diameter of the shell, diameter of the mouth of the shell, and, 3) in A. polypora, the number of nuclei, size of the chromatin bodies within the nuclei, diameter of the shell, and diameter of the mouth of the shell. A correlation was also demonstrated between the diameter of the shell and the diameter of the mouth of the shell in this species.

6. The isolation of heritably diverse lines in Arcella polypora within a family during vegetative reproduction

It has been shown by Jennings ('16) in Difflugia, by Middleton ('15) in Stylonychia, by Root ('18) in Centropyxis, and by Hegner ('19) in Arcella dentata, that by selection, heritably diverse lines may be isolated within a family during vegetative reproduction. No extended attempt was made to discover whether

this also holds true for other species of Arcella, but evidence was obtained that indicates that no difficulty would be experienced in securing similar results with A. polypora. In family ap. 34 two lines were noted which contained specimens with the same number of nuclei (7), but whose mean diameter showed a difference of 3.28 units of 4.3 μ each. It was not possible to carry on these lines, and so only a few specimens were reared (thirty-seven in all), but even this small number is significant in the light of the data we already possess in other species of Protozoa.

7. *Chromidia*

Arcella is one of the first organisms in which extra-nuclear granules, now known as chromidia, were discovered. A band of these granules was described by R. Hertwig in 1887, and the same investigator twelve years later (Hertwig, '99) recorded the formation of secondary nuclei from this 'extra-nuclear chromatin net.' These nuclei, according to Elpatiewsky ('07), become the centers of amebulae of two sizes, which are macrogametes and microgametes and which conjugate in pairs. The conjugation of the entire chromidial nets of pairs of Arcellas was reported the following year by Swarczewsky ('08). Chromidia have been described in many groups of Protozoa and seem to play an important part in their life cycle. For this reason it seems worth while to mention the apparent lack of influence of this extra-nuclear chromatin during the bisection experiments performed on Arcella dentata. These experiments demonstrate that when from one-tenth to three-fourths of the entire chromidial net is removed, the descendants of the part that remains invariably attain the normal condition of the line. When a large Arcella is bisected and both nuclei are present in one-half, the other half without nuclei always dies within a few days without any visible attempt on the part of the chromidia to form new nuclei. This is true even when only the nuclei are removed and all the cytoplasm is left in the shell. The evidence justifies the conclusion that the chromidia play no rôle in vegetative reproduction and at this time have no influence upon the size of the organism nor upon

the characteristics of the shell. No stages appeared in my cultures resembling those described by the investigators mentioned above, so it was impossible to determine whether the removal of chromidia has any effect upon the formation of secondary nuclei and upon the characteristics of the generations that appear after conjugation.

8. The relations between internal changes and external characteristics

One of the most striking results of the cutting experiments performed on Arcella dentata is the discovery that a large internal change is only slowly expressed by the external characters. Thus, when a uninucleate specimen becomes binucleate, its subsequent offspring, which are also binucleate, do not at once become as large nor possess as many spines as normal binucleates, but show only a small increase in these respects over their parent. Additional increases in the second, third, and fourth generations, however, finally lead to the attainment of the normal binucleate condition, and when this state is reached no further increase takes place.

This gradual change following nuclear doubling looks very much like variation in a definite direction (orthogenesis), and, if the nuclei could not be seen, as is the case in most of the other shelled rhizopods, one might interpret the results as due to successive internal changes rather than to a single modification. The writer believes that heritable variations are all discontinuous, but that they differ from one another in degree. The reactions of Arcella to internal changes indicate, however, that unless nuclear conditions are closely followed, it is impossible to determine in shelled rhizopods and other similar organisms whether gradual variations in a definite direction are due to small or to large internal differences. Such variations, therefore, as those observed by Jennings in Difflugia and by Root in Centropyxis may have been due to abrupt changes in the nuclear condition that were gradually being expressed in successive generations by the external characters. That is, they were really what are generally known as mutations and not variations of the Darwinian type.

9. The effects of selection upon the production of heritably diverse lines in shelled rhizopods

Jennings ('16), Root ('18), and the writer (Hegner, '19) have all found it possible by selecting certain specimens within a line produced by vegetative reproduction to obtain branch lines that differed from one another in heritable characters. In neither Difflugia, upon which Jennings worked, nor Centropyxis which Root used for his researches are the nuclei visible in the living animal. These investigators did not know, therefore, whether nuclear changes occurred in their organisms or not. The heritable diversities reported by Jennings took place principally by gradual variations, but sudden changes (mutations) also appeared. Both sorts of variations may have been due to changes in the nuclear number, the effects of which were in certain cases slowly and in other cases more quickly revealed by the external characters. In the selection work carried on by the writer on Arcella dentata, the nuclear condition of the organisms was under constant observation, and, although empty shells appeared, no specimens with more than two nuclei were found and only a very few with one nucleus were discovered. The heritable diversities that were obtained were, therefore, not due to changes in nuclear number. There is evidence, however, that they may have been due to changes in the quantity of chromatin contained in their nuclei. Unfortunately, these diverse branch lines were allowed to die out before it was discovered that differences in chromatin mass existed in the different pure lines. As shown in part 6 of this contribution, the size of the members of a line of Arcella dentata is correlated with the quantity of chromatin in their nuclei, and the chromatin mass in specimens belonging to a line containing small members is less than in a line composed of large specimens. It is, therefore, possible that the diverse branch lines resulted from inequalities in the distribution of the chromatin masses to the daughter nuclei during fission. Diameter of shell and spine number are closely correlated in these organisms (Hegner, '19), and thus when spine number was used as a basis for selection, size was also involved. Similarly, since size and chro-

matin mass are correlated, it follows that spine number and chromatin mass are likewise correlated. It is possible, therefore, that although diverse lines were isolated by the selection of specimens according to their spine number, what was really being selected was variations in the chromatin mass within the nuclei. The permanency of these diverse branch lines consequently depends upon the permanency of the differences in chromatin mass. It does not seem probable that an unequal quantitative division of the chromatin could bring about the permanent diversities that were obtained, since the formation of new chromatin is a regular process during the growth of the cell, and any inequality in chromatin mass following cell division would soon be overcome by the growth of the chromatin. It has been suggested by Jennings in Difflugia that "the substances determining the hereditary characters may be distributed with less accuracy than in higher organisms, so that the two products of fission may often receive parts that are not equivalent" (Jennings, '16, p. 524). This may likewise be true of Arcella. The sudden large heritable changes (mutations) would, according to this suggestion, be due to large qualitative inequalities and the smaller heritable variations to smaller qualitative inequalities during nuclear division.

We may recognize in these organisms both fluctuating and heritable variations, but it is of course impossible to distinguish immediately from the shell characteristics which of these two variations is being selected, and hence the rate of isolation of heritably diverse branch lines is, according to the laws of chance, less rapid than it would be if the two types of variations could be determined at once.

With one exception, all of the characters of Arcella that were studied were found to vary together, the correlation being very high. In Difflugia, however, Jennings worked with several characters that varied independently. Thus "there is some indication in the pedigrees that hereditarily higher numbers of spines need not necessarily go with hereditarily larger size, though they usually do" (Jennings, '16, p. 519), and "Hereditarily diverse combinations of size and length of spines occur in the different

branches" (p. 520). The length of the spines of Arcella dentata did not seem to the writer to be a favorable character for selection work and hence was neglected in favor of spine number and diameter of the shell. Great variations in length of spine were frequently encountered, but they were so obviously due to the effects of environmental factors that it was not considered advisable to undertake a study of them from the selection standpoint. As we have seen, there appear to be among the groups of Arcella polypora at least two characters that vary independently, i.e., the diameter of the shell and the diameter of the mouth of the shell. Selection work with these characters would probably prove them to be independent and would thus furnish a case similar to that noted by Jennings in Difflugia.

How can we interpret these independently varying characters in the light of our investigations on Arcella? Nothing, of course, can be stated with certainty, but it may be worth while to make suggestions. If the assumption is correct that the daughter nuclei formed during vegetative reproduction are qualitatively different, then there is no reason why all of the characters of the organism should be correlated. Adopting the current theory that the determiners for different characters are represented in the nuclear chromatin by different molecules or group of molecules, there seems to be no reason why in these organisms inequalities may not occur independently in the different determiners resulting in corresponding inequalities in the different external characters. If the external characters are all correlated, we may assume that their determiners are linked in some way that leads to similar interdependent variations.

It is, of course, possible that independent characters are represented within the chromatin by multiple factors and that the inequality hypothecated consists not in an unequal division of one determiner but in the unequal distribution of a number of determiners to the daughter nuclei.

The cutting experiments on Arcella dentata lessen the possibility that the cytoplasm plays an important rôle in the production of the heritable diversities observed. The chromidia as a factor in this process seem also to be ruled out by these experi-

ments. The final conclusion is, then, that the size of the organism and the characteristics correlated with size are dependent upon the chromatin mass; that changes in these characters are not due to cytoplasmic nor chromidial influence, but to qualitatively unequal nuclear divisions, resulting in two types of daughter nuclei differing in the determiners that control the growth of the chromatin, and that other characters that vary independently must be controlled by other determiners within the nuclei.

Summaries of results will be found on pages 31, 59, 65, and 76.

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Resumen por los autores, Clarence N. Jackson y Chester A. Stewart
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Los efectos de la inanición en el animal jóven sobre el tamaño definitivo del cuerpo y de varios órganos de la rata albina

En el presente trabajo los autores han empleado treinta y ocho crías; 113 ratas sobrevivieron a los experimentos, 35 machos y 35 hembras experimentadas y 27 machos y 16 hembras empleados para comparación. Los grupos de ratas fueron sometidos a una alimentación insuficiente desde el nacimiento a las 3, 6 y 10 semanas de edad, y desde las 3 a las 20 semanas, es decir, casi un año. Una vez transcurridos los periodos mencionados se las dió alimento en abundancia. Crecieron de un modo variable, pero permaneciendo siempre imperfectamente desarrolladas, sin llegar a alcanzar el tamaño de los individuos adultos empleados como tipo de comparación. Stewart (1916) comprobó el restablecimiento completo de los animales así tratados después de estar sometidos a una alimentación insuficiente durante 3 a 10 semanas. El efecto final varía por consiguiente en relación con la edad del animal y la duración del periodo de inanición. Esto concuerda con los resultados obtenidos por Aron y Brüning, pero no con los obtenidos por Osborne y Mendel. Cuarenta y cinco ratas (28 experimentadas y 17 empleadas como comparación) fueron autopsiadas. Los órganos de los animales sometidos a los experimentos se compararon con los de animales normales del mismo peso. Las longitudes del cuerpo y de la cola son ligeramente subnormales en tales animales; la cabeza, miembros y tronco presentan casi el peso normal; el esqueleto, tegumento y la musculatura son ligeramente subnormales, el grupo visceral un poco por encima de lo normal, y el "resto" varía. De los órganos, considerados individualmente, el cerebro, médula espinal, hipófisis y pulmones son en general ligeramente subnormales, mientras que los ovarios son claramente subnormales. El corazón y el tubo digestivo pesan un poco mas que lo normal, mientras que los testículos y epidídimos aumentan de peso de un modo bien manifiesto. Mientras que aparecen de este modo algunas anormalidades, son generalmente de poca importancia, y en general los órganos y partes del cuerpo son casi normales en las ratas imperfectamente desarrolladas en toda su vida. Por consiguiente la inanición en las primeras edades retarda aparentemente el proceso del crecimiento ulterior del conjunto del cuerpo.

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THE EFFECTS OF INANITION IN THE YOUNG UPON THE ULTIMATE SIZE OF THE BODY AND OF THE VARIOUS ORGANS IN THE ALBINO RAT

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FIVE CHARTS

The question as to the effects of malnutrition during infancy and youth upon the capacity for later growth is of especial interest at present, on account of the prevalence of famine in connection with the world war. It is generally believed that early starvation may result in permanently stunting the later growth of the body, although accurate data for the human species are lacking. The results of animal experiments on this subject are somewhat contradictory. Aron ('10, '11, '14) and Brüning ('14) found that severe underfeeding of young dogs and rats apparently prevents them from reaching normal adult size upon later full feeding. On the other hand, Hatai ('07) and Stewart ('16) obtained complete recovery upon re-feeding rats which had been underfed for short periods beginning at three or four weeks of age. Osborne and Mendel ('14, '15) also found no suppression of growth capacity in young albino rats whose growth had been retarded for long periods by various inadequate diets. The object of the present investigation is to determine the ultimate effect of such underfeeding when begun earlier (in the new-born) or when prolonged over a very extensive period. The study is also extended to include the effects upon the final weights of the various component parts, systems, and organs of the body.

MATERIAL AND METHODS

The white or albino rat (Mus norvegicus albinus) was used in the experiments, for which it is in many respects well adapted. It is hardy and thrives in captivity. It breeds rapidly (period of gestation three weeks), reaches sexual maturity at ten weeks, and adult size within a year. The normal growth rate and variability of the albino rat and its various organs have also been very thoroughly worked out, chiefly by Donaldson, Hatai, Jackson, and Lowrey, and normal growth tables by Donaldson ('15) are available for comparison. Moreover, the effects of acute and chronic inanition upon the weight of the body and of the various organs in the rat at different periods have been studied by Jackson ('15, '15 a), Stewart ('16, '18, '19), and Jackson and Stewart ('18, '19). The data available from these studies make possible a comprehensive view of the effects of inanition at various ages and the rapidity of recovery upon refeeding in an organism whose normal growth rate and variability are well known. The lack of such data frequently makes it impossible to draw trustworthy conclusions from the results of experimental work, especially when the observations are limited in number.

In the present investigation, thirty-eight litters of albino rats from the colony in The Institute of Anatomy were used. About half of the rats died from starvation during the experiments and are excluded from consideration. One hundred and thirteen rats survived, including 35 male and 35 female test rats, with 27 male and 16 female normal controls. In general, test rats and controls were available from each litter, as shown by the data in table 1. In this table, the letters 'St' (Stewart) refer to the series to which the rat belonged, the number preceding the decimal point indicates the litter, and the number following designates the individual. The final letter 'm' indicates male, or 'f,' female.

As further shown in table 1, the experiments may be divided into (A) those in which the preliminary underfeeding began at birth, and (B) those beginning at three weeks of age. The rats

in division A were underfed by removal from the nursing mother at successive intervals, as described by Stewart ('18). In eight litters, the test rats, which weighed about 5 grams at birth, were retarded so as to reach only about 10 or 12 grams (the normal being 20 to 25 grams) at three weeks of age. After this they were fully fed, as were the controls throughout. They received (after the weaning period) an abundant diet of whole wheat (Graham) bread soaked in whole milk. Water from the city supply (Mississippi River) was given ad libitum throughout. In one litter (no. 86) the underfeeding was similarly prolonged from birth to four weeks of age, in three litters to six weeks, and in nine litters to about ten weeks (the extreme for this group being eighty-three days). The underfed rats at ten weeks (beginning of refeeding) had reached only about 15 or 16 grams in body weight, the normal at that age being over 100 grams.

In division B, the underfeeding did not begin until the age of three weeks (the weaning period), at a body weight of 20 to 25 grams. In one litter (no. 33) the test rats were refed at twenty weeks (138 days), the body weights then being 33 to 43 grams. In the remaining sixteen litters, the underfeeding was prolonged to nearly a year of age, the average being 342 days (range 235 to 375 days). The body weights of the test rats at this time (beginning of refeeding) averaged about 60 grams, while the normal controls had reached their maximum adult size (about 200 grams in the females and 250 grams in the males).

The curves of the average body weights in the various test and control groups (sexes separately) are shown in charts 1 to 5. The composite curves were constructed from the average body weights for the individuals of each group at corresponding ages, excepting in the refed rats. Since the test rats in each group were not all refed beginning at the same age, the average date of the beginning of refeeding in each group was taken as the starting point for the refed animals, and their body weights arranged and averaged according to the number of days of refeeding in each case.

The rats were well cared for throughout the experiments, being kept during underfeeding in a warm room on account of unusual susceptibility to cold at this time. They were weighed daily at the beginning of the underfeeding and the refeeding periods, the time of weighing being later extended to intervals of several days. The males and females of the same litter were usually not separated, and the number of litters born to each female is recorded in table 1. While no care was taken to keep males with the females constantly in all cases (and hence the number of litters is not the maximum possible), it may be stated that the test rats had at least as good an opportunity for breeding as did the controls.

At the end of the experiments, the rats either died or were killed, as indicated in table 1. In forty-five cases (28 test rats and 17 controls) autopsies were performed, and the weights of the various parts, systems and organs recorded. The technique used was the same as described in the earlier papers by Jackson and Stewart. In this series, Stewart was responsible for the care of the animals and the performance of the autopsies.

The data for the organ weights (also body length and tail length) are given in table 2 under four groups: those refed after underfeeding from, 1) birth to 3 weeks; 2) birth to 10 weeks; 3) 3 weeks to 20 weeks of age, and, 4) 3 weeks to nearly a year (average 342 days) of age. The controls in each group are not all from the same litters as the test rats, some being added (selected from the other groups) to make the average body weight of the controls about equal to that of the corresponding test rats in each group. In the fourth group, however, controls were not available so low in body weight as the test rats, and a different mode of comparison was used. For the body parts and systems of this group data from Jackson and Lowrey ('12) were used for the normal in rats of corresponding body weight. For the individual organs, the control rats (four males, eight females) from the corresponding litters were used, although they were much heavier than the test rats. Following a method suggested by Donaldson ('15), both controls and test rats were compared with the Wistar norm for rats of the same body weight

(rather than body length), and the percentage difference established for each organ. The algebraic difference between these percentage results for the control and the test rat in each case is taken as indicating the percentage of change in the corresponding organ. Therefore, the average percentage changes for the organs in the fourth column in table 1 are calculated by a method different from that in the first three columns of the table, where the absolute data for test rats and controls are directly compared. In making the comparisons of organs, the very slight differences in body weight (not exceeding 2 per cent) between test rats and controls in each group were ignored.

For economy of space, only the average data are given in table 2. The sexes were at first grouped separately, but were later combined in all cases excepting those with marked sexual difference in weight (hypophysis, suprarenals, and gonads). The individual data will be filed later in The Wistar Institute of Anatomy, Philadelphia, where they may be obtained if desired.

On account of the relatively small number of observations, it is evident that the results for the various organs may be influenced by individual variation and experimental error, hence they are not to be considered final. Where the changes are marked and constant, however, they indicate the result with a considerable degree of probability; and vice versa, when the tests and controls differ but slightly, we may assume it as probable that the earlier inanition has had no marked effect upon the relative or proportional size of the organ at the end of the refeeding period.

EFFECTS ON BODY WEIGHT

The effects of the underfeeding and subsequent refeeding upon the body weight in the various groups are shown by the curves in charts 1 to 5. Chart 1 shows the composite curves for the eight litters underfed from birth to three weeks of age, including 11 test males, 12 test females, 6 control males and 4 control females. The normal difference in weight according to sex is evident. The sudden rise in the curves for the controls at about 300 days of age is due to the death of individuals

with abnormally low body weights, causing previous depression of the curves. Although in five of the twenty-three test rats (table 1) the maximum body weight reached or exceeded slightly that of the controls of the same sex in the same litter, the average for the test rats for each sex remains definitely below that of the controls.

The curves of body weight for the rats refed after underfeeding from birth to six weeks of age are shown in chart 2. This is a small group, the males including 1 test and 1 control rat from litter St. 61, the females including 2 test and 2 control rats from litters 52 and 71 (table 1). Although the normal maximum body weight was not reached by either male or female controls (in the former, on account of a wounded foot), they remain at all times above the test rats of corresponding sex, as shown by the chart and by table 1.

In chart 3 are shown the curves of body weight for the rats refed after underfeeding from birth to about ten weeks (average 72 days) of age. They include nine litters, with 10 test males, 9 test females, 8 control males, and 3 control females (table 1). The sudden rise in the curve for control males at 490 days is not due to an actual increase in body weight, but to the survival of a single rat of unusually large size. Similarly in the test females, the continued rise in body weight after 340 days is due partly to the elimination of one rat (St. 111.8) which was very low in final weight, and to the survival of an unusually large test rat (St. 111.4). The final gross body weight of the latter (212 grams) was 1 gram above that of the surviving control (St. 111.5). In no other case did the maximum body weight of a test rat in this group reach that for the control of the same sex in the same litter. Even in this case, the net body weight (gross weight minus intestinal contents) of the test rat was 191 grams, while that of the control was 198 grams. Both were pregnant when killed. Except at this point, the average body weights for the test animals, as shown by the chart and by table 1, remain definitely below those for the controls of the same sex.

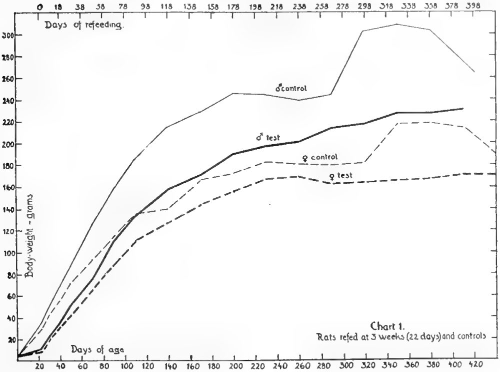


Chart 1.
Rats refed at 3 weeks (22 days) and controls

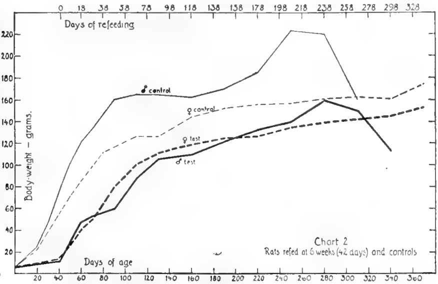


Chart 2
Rats refed at 6 weeks (42 days) and controls

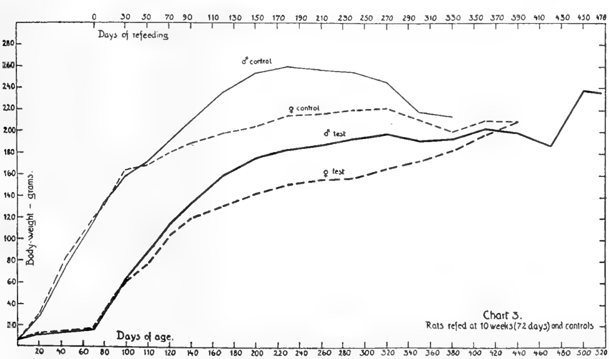
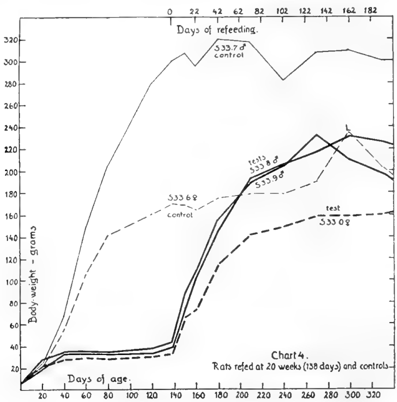
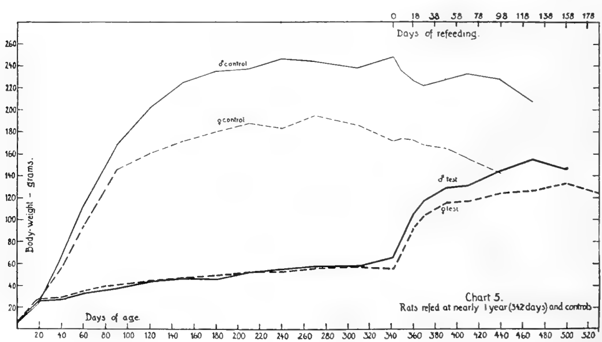


Chart 3.
Rats refed at 10 weeks (72 days) and controls

Chart 4 represents the individual curves of body weight for five albino rats from litter S33, refed after underfeeding beginning at three weeks and extending up to twenty weeks (138 days) of age. The test rats included 2 males and 1 female,





with 1 male and 1 female full-fed control (table 1). 'L' indicates the birth of a litter by the female control. While the test rats grew rapidly for awhile after refeeding, the growth soon ceased and their permanent depression in body weight, in comparison with that of the controls, is obvious.

TABLE 1

Individual data for the gross body weight of normal controls and test rats refed after underfeeding for various periods

RAT NUMBER AND SEX	REFED AT		MAXIMUM WEIGHT	FINAL		TERMINUS * = AUTOPSY	NUMBER OF PREGNANCIES
	Age	Weight		Weight	Age		
A. Rats underfed from birth, and normal controls							
	days	*grams*	*grams*	*grams*	*days*		
St 134.1 m	(Control)		236	226	288	Died	
St 134.3 m	21	10.3	193	159	330	Died	
St 134.5 f	21	10.6	151	141	395	*Killed	0
St 134.6 f	21	10.7	174	167	452	*Killed	0
St 122.2 m	(Control)		345	337	380	*Killed	
St 122.8 m	22	10.8	230	224	409	*Killed	
St 122.6 f	(Control)		248	231	422	*Killed	3
St 122.1 f	22	10.5	208	186	422	*Killed	3
St 49.1 m	(Control)		273	239	270	(?)	
St 49.2 m	22	11.0	180	179	270	Died ?	
St 49.3 m	22	11.6	176	173	270	Died ?	
St 49.4 f	22	13.0	180	125	216	Died	2
St 70.5 m	(Control)		188	133	321	Died	
St 70.1 m	22	11.5	186	170	302	Died	
St 70.2 m	22	11.3	202	174	356	Died ?	
St 70.3 f	(Control)		208	190	440	Alive	2
St 70.4 f	22	12.5	201	158	440	Died	2
St 125.4 f	(Control)		173	156	323	Died ?	0
St 125.2 f	22	9.8	167	165	323	Died ?	0
St 125.5 f	22	9.0	173	168	488	Died	0
St 140.7 m	(Control)		244	244	147	Died	
St 140.8 m	21	9.0	218	207	388	Died	
St 140.3 f	(Control)		177	173	325	Died	1
St 140.1 f	21	10.0	192	176	458	Alive	0
St 140.5 f	21	9.5	201	181	458	Alive	1
St 148.3 m	(Control)		287	264	423	Alive	
St 148.1 m	21	9.2	130	130	197	Died	
St 148.7 m	21	9.5	269	243	423	Alive	
St 148.8 m	21	9.9	252	222	423	Alive	
St 152.6 m	21	9.1	177	177	184	Died	
St 152.2 f	21	10.2	185	156	400	Alive	1
St 152.5 f	21	10.5	187	160	400	Alive	0
St 152.8 f	21	10.5	166	166	184	Died	1
St 86.5 m	(Control)		298	215	446	Died	
St 86.2 m	28	13.0	206	184	549	*Killed	
St 61.5 m	(Control)		223	154	314	Died ?	
St 61.3 m	42	12.2	160	113	341	Died ?	
St 71.7 f	(Control)		175	175	369	Alive	2
St 71.2 f	42	13.2	158	153	369	Died	0

TABLE 1—*Continued*

RAT NUMBER AND SEX	REFED AT		MAXIMUM WEIGHT	FINAL		TERMINUS * = AUTOPSY	NUMBER OF PREGNANCIES
	Age	Weight		Weight	Age		
A. Rats underfed from birth, and normal controls							
	days	*grams*	*grams*	*grams*	*days*		
St 52.7 f	(Control)		160	152	345	*Killed	0
St 52.4 f	42	12.2	140	130	345	*Killed	0
St 146.7 m	(Control)		295	220	340	Died	
St 146.5 m	60	16.0	205	172	408	Alive	
St 146.6 m	60	16.0	251	203	441	Alive	
St 145.2 m	(Control)		251	246	410	Died	
St 154.4 f	61	16.8	174	167	410	*Killed	1
St 145.5 f	61	16.0	188	183	410	*Killed	1
St 76.6 m	(Control)		280	251	357	Alive	
St 76.8 m	(Control)		310	260	357	Alive	
St 76.6 m	70	15.6	171	142	357	Alive	
St 76.9 f	(Control)		238	234	357	Alive	4
St 76.10 f	70	17.0	152	140	357	Alive	0
St 73.6 m	(Control)		187	187	180	Alive	
St 73.7 m	70	12.7	103	103	153	Died	
St 73.2 f	70	16.5	87	87	153	Died?	0
St 65.6 m	(Control)		217	167	291	Died	
St 65.4 m	70	14.9	158	135	500	Died	
St 65.9 m	70	14.9	138	138	186	Died	
St 65.10 f	(Control)		196	196	174	Died	0
St 65.2 f	70	15.0	117	117	186	Died	0
St 138.6 m	(Control)		283	223	390	Alive	
St 138.1 m	70	15.6	208	149	357	Died	
St 111.3 m	(Control)		247	115	448	Died	
St 111.9 m	69	16.0	243	236	536	*Killed	
St 111.1 m	69	15.5	182	182	321	(?)	
St 111.5 f	(Control)		211	211	465	*Killed	4
St 111.8 f	69	16.0	181	149	351	Died?	3
St 111.4 f	69	13.8	212	212	465	*Killed	4
St 173.1 f	80	17.0	136	136	326	Alive	0
St 173.3 f	80	16.0	126	120	326	Alive	0
St 180.5 m	83	16.5	237	237	315	Alive	
B. Rats underfed from age of three weeks, and normal controls							
S 33.7 m	(Control)		316	307	346	*Killed	
S 33.9 m	138	43.0	236	191	335	*Died	
S 33.8 m	138	38.5	233	229	346	*Killed	
S 33.6 f	(Control)		233	194	339	*(?)	1
S 33.0 f	138	32.5	162	162	339	*(?)	0
S 16.0 m	(Control)		243	176	261	*Died?	
S 16.1 m	255	53.0	119	94	368	Died	

TABLE 1—*Concluded*

RAT NUMBER AND SEX	REFED AT		MAXIMUM WEIGHT	FINAL		TERMINUS * = AUTOPSY	NUMBER OF PREGNANCIES
	Age	Weight		Weight	Age		
B. Rats underfed from age of three weeks, and normal controls							
	days	*grams*	*grams*	*grams*	*days*		
S 16.6 f	(Control)		162	158	275	Alive	3
S 16.4 f	255	58.0	127	111	321	Died	0
St 20.4 m	(Control)		263	183	474	Died	
St 20.8 m	330	60.5	125	114	493	*Died	
St 38.3 m	(Control)		210	197	288	Died	
St 38.4 m	331	63.0	187	168	519	*Killed	
St 38.6 f	331	45.0	120	100	443	*Killed	0
St 32.5 f	358	58.0	114	108	490	*Killed	0
St 41.6 m	(Control)		293	270	310	*Killed	
St 41.4 f	310	52.5	137	100	399	*Died	2
St 19.8 f	(Control)		201	151	305	*Killed	4
St 19.5 f	331	78.5	130	121	532	*Killed	0
St 25.8 m	(Control)		281	228	298	Died?	
St 29.7 m	(Control)		290	197	365	*Died	
St 29.2 m	331	59.0	157	143	492	*Killed	
St 29.9 f	(Control)		184	168	188	*Died	1
St 29.6 f	331	54.0	129	119	436	*Died	0
M 9.11 m	(Control)		285	213	400	Died	
M 9.9 m	332	72.0	171	119	425	Died	
St 46.9 m	335	48.0	158	146	489	Alive	
St 46.2 f	335	56.3	157	157	489	Alive	0
St 46.5 f	335	52.6	98	98	412	Died	0
St 36.2 m	(Control)		267	255	475	*Killed	
St 36.3 m	366	69.5	118	85	442	*Died	
St 33.1 m	(Control)		252	196	493	*Killed	
St 33.4 m	375	61.0	167	147	529	*Killed	
St 34.7 m	(Control)		241	199	401	*Killed	
St 34.6 m	374	52.4	181	154	484	*Killed	
St 35.2 m	368	56.5	122	119	418	*Died	
St 35.6 f	(Control)		171	141	477	*Killed	3
St 35.4 f	368	60.5	146	125	501	*Died	0
St 35.5 f	368	52.0	141	125	524	*Killed	0
St 35.7 f	368	45.0	104	91	438	*Died	0
St 24.7 f	(Control)		210	181	344	*Died	1
St 26.9 f	(Control)		235	197	343	*Killed	3

In chart 5, the average body weight curves are shown for the albino rats refed after underfeeding from three weeks up to nearly a year (average 342 days) of age. Rats from sixteen litters are included, with 10 test males, 11 test females, 10 control males, and 6 control females. In no case did the maximum body weight of an individual test rat approach that of the corresponding control of the same litter and sex (table 1). As shown by the curves, the underfed rats grew rapidly for a time after refeeding, but were permanently stunted and unable to attain the normal adult body weight, although amply refed until growth had ceased.

While there is clearly a permanent stunting in the body weight of the rats as a result of the earlier period of underfeeding, it is difficult to measure the amount of depression on account of irregularities in the various curves, as shown in charts 1 to 5. Measured roughly, however, it may be said that the shortage in ultimate body weight in the test rats varies from about 10 per cent in chart 1 to about 35 per cent in chart 5. We may consider the dwarfing effect of inanition in general as varying according to, 1) the length of the underfeeding period; 2) the age at which the inanition occurs; 3) the sex; 4) the severity, and, 5) the character of the inanition.

As to the length of the inanition, it is found, as might be expected, that in general the longer the period of starvation in the young animal, the more marked is the depression in ultimate body weight. This is not so clear in charts 1, 2, and 3, as in charts 4 and 5. The curves of Stewart ('16) show complete recovery of rats fully refed after a period of maintenance (at constant body weight) by underfeeding beginning at three weeks and extending up to four, six, or ten weeks of age. (The males at ten weeks formed an exception, which will be considered later). The present experiments show that if the underfeeding beginning in rats at the same age (three weeks) is extended up to twenty weeks, even allowing a moderate increase in the body weight, there is a marked permanent depression of the ultimate body weight, as shown in chart 4. If the underfeeding is prolonged to nearly a year, the effect is still greater,

the ultimate body weight of the refed survivors being more than one-third below that of the normal controls.

This result is in general agreement with Aron ('14), who found that if the underfeeding of young rats is prolonged beyond 50 to 150 days, the rats upon full refeeding fail to reach their normal size and weight, thus remaining permanently stunted or dwarfed. Aron therefore concluded that full recovery upon refeeding is possible only when the retardation of the growth has not extended beyond the time of the normal active growth period.

Brüning ('14) found that young nursing rats underfed by removal from the mother for successive periods are greatly retarded in later growth. The normal body weight was not fully recovered upon subsequent full feeding, but the experiment was not continued long enough to determine whether the effect was permanent.

Hatai ('07), however, observed complete recovery of normal body weight on refeeding after partial starvation (starch diet) for three weeks in rats one month old. Stewart ('16) also obtained similar results in rats refed after maintenance (constant body weight) by underfeeding from three to ten weeks of age.

Osborne and Mendel ('14, '15), in an extensive series of experiments upon the growth of rats with various inadequate protein diets, likewise found a remarkable capacity for full recovery of body weight upon proper refeeding, even after growth has been suppressed for periods of time (up to 558 days) far beyond the normal growth period. They claim that the capacity to resume growth does not depend upon the size or age at which the inhibition of growth is effected, and state that: "It is now reasonable to ask whether the capacity to grow can ever be lost unless it is exercised." A considerable number of cases, with growth curves, are presented to support this conclusion. These are chiefly rats in which growth was retarded by qualitatively deficient (inadequate protein) diets, which will be discussed later. In one case, however, a female rat in which growth was repressed by 'limited quantity of food' (no further details given), the body weight of 53 grams at 39 days of age reached

only 59 grams at 513 days. Rapid growth ensued upon full refeeding, the body weight reaching 220 grams, which is above the normal maximum, in about 130 days. Unfortunately, no direct controls from the same litters were kept in the experiments of Osborne and Mendel. They admit that "resumption of growth has not been as perfect in every case as in the typical records here presented," but the positive results are considered more valuable, since failure may be due to various causes. Their results will be referred to again later.

As to the variation in the effect of inanition according to the age of the animal, it is evident from charts 1 and 2 that underfeeding beginning at birth produces permanent stunting, whereas Stewart ('16) found that an equal (or greater) degree of underfeeding for similar periods beginning at three weeks of age is followed by complete recovery of body weight upon later full refeeding. As previously mentioned, Brüning likewise apparently obtained a permanent retardation of growth of nursing rats. It is not strange to find that a permanent stunting is more readily obtained in younger animals (probably likewise in the human species), when the normal growth process is most active and the organism in general is less resistant to starvation. The greater power of recuperation found by Osborne and Mendel is probably due in part to the fact that their rats were underfed beginning usually at five weeks or more of age, at which time their resistance to inanition is greater.

In this connection it is of interest to note that both Dunn ('08) and King ('16) found that rats greatly undersized at birth, although they may grow vigorously for a time, usually fail to reach the normal adult size. In these cases, King assumes that "The normal action of growth factors is inhibited from the very beginning of postnatal life by unknown constitutional causes, not by environmental conditions." It seems to be quite possible that in such cases the small size at birth may be due to prenatal malnutrition, which might be expected to exert a more detrimental effect during the earlier stages of the organism.

As to sex, it appears from the present experiments that in general the depression in permanent body weight by early inanition is relatively greater in the males than in the females. This is evident in all the charts, 1 to 5. It is also in agreement with the observation by Stewart ('16), who found that in rats held at maintenance by underfeeding from three to ten weeks of age, the females made full recovery upon refeeding, while the males apparently did not. On the other hand, there is no evidence to indicate that the mortality is markedly greater among the males during the inanition period, since the survivors of the experiments are about equally distributed according to sex.

Since the ultimate body weight is affected by the bearing of young (Watson, '06), this factor must be considered. The pregnancies occurring in each group of the present series are given in the following table:

EXPERIMENT	TEST FEMALES			CONTROL FEMALES		
Refed after underfeeding from	Number of rats	Number pregnant	Total number of pregnancies	Number of rats	Number pregnant	Total number of pregnancies
Birth to 3 weeks	12	6	10	4	3	6
Birth to 6 weeks	2	0	0	2	1	2
3 to 10 weeks	9	4	9	3	2	8
3 to 20 weeks	1	0	0	1	1	1
3 weeks to 1 year	11	1	2	6	6	15
Total	35	11	21	16	13	32

It is evident from the table that although opportunity for breeding was equally good in the test rats, their reproductive capacity was very decidedly reduced. This is especially striking in the last two groups, where only one of the twelve test females became pregnant at all; whereas all of the seven control females became pregnant, bearing a total of sixteen litters.

Watson ('05) found that the body weight of female albino rats bearing young (three litters) averages about 9 per cent greater than in unmated female controls. This agrees closely with the results of Minot ('91) for guinea-pigs. This might be sufficient to account for the difference between the test and the control

females in charts 1, 2, and 3, if all the controls had borne young and all the test rats were virgins. As shown in the table above, however, this was not the case, although pregnancies were considerably more frequent in the control rats. In the rats which had undergone prolonged inanition (charts 4 and 5), pregnancy occurred very rarely, as above shown, but in these the excess weight of the control is far greater than could be explained by their bearing of young. It is evident, however, that if the female control rats had borne no young their weight would have been slightly less and therefore somewhat nearer to that of the test rats. The contrast between the results in male and female emphasizes the difference between the sexes in their depression of ultimate body weight as a result of an early period of underfeeding.

The question as to whether the dwarfing of the body produced by inanition could be transmitted by heredity (in the few cases where reproduction occurs) is of considerable interest. In the present series, however, records are available only in the case of the one female (St. 41.4) which bore young after the long underfeeding period (3 weeks to 310 days). This extreme case might be expected to show such an effect if any were possible. This female reached a maximum of only 138 grams in bodyweight. Mated with a normal male (of the same litter), she bore two litters. One of these, litter no. 133a, was kept under observation. The litter included four males and two females, of normal size at birth. One male died early; the others reached adult maximum body weight of 215, 226, and 244 grams. The two females reached maximum of 174 grams and 204 grams, both being pregnant at the time. Thus they were slightly below the normal maximum weight, and each bore only one litter, but this is not unusual even among the control rats (table 1). The two females and one male were alive and apparently normal at fourteen months of age. The results therefore do not indicate the transmission of any marked hereditary effect of inanition, though further observations would be necessary to establish any definite conclusion on this point. The result might of course be slight, but cumulative in successive generations.

There is also unquestionably a difference in the effect of underfeeding upon the ultimate growth of the body, depending upon the severity of the inanition. As is well known, short periods of inanition, even sufficiently severe to repress growth temporarily, do not prevent the attainment of full adult size on subsequent refeeding (Hatai, '07; Stewart, '16). The favorable results obtained by Osborne and Mendel ('14, '15, '17) upon refeeding after longer periods of inanition may be due in part to the fact that in most cases their rats were not only older at the beginning of the experiment, but were allowed to increase during the experiment to a body weight considerably greater. That is, the inanition was somewhat less severe than in our present series. This perhaps also accounts for the fact that Osborne, Mendel and Ferry ('17) found no decrease of reproductive capacity in their female rats when amply refed after an extensive earlier period of stunting.

It should be emphasized that the present experiments represent very extreme degrees of underfeeding, at which even with the greatest care many of the rats perish from inanition. Thus the rats underfed from birth so as to restrict their body weight to 15 or 16 grams at ten weeks would be roughly comparable to children about twelve years of age so stunted by underfeeding from birth as to reach a body weight of about 20 pounds, normally reached at one year. Similarly, a rat underfed from three weeks to nearly a year (340 days) of age at about 60 grams body weight would correspond roughly to a human of adult age dwarfed by underfeeding from infancy so that the body weight would not exceed that of a normal child below the age of puberty.

Finally, there is doubtless a difference in the effect according to the character of the inanition, particularly in inanition due to qualitative deficiencies in the diet. Aron ('14) found that a protein-poor diet appeared to affect more unfavorably the subsequent growth of rats upon later refeeding than did a corresponding degree of underfeeding with restricted amounts of a normal diet. On the contrary, as previously mentioned, Osborne and Mendel ('14, '15) obtained complete recovery of body weight upon proper refeeding of albino rats in which growth had

been almost completely suppressed for long periods by various inadequate protein diets. It is quite possible that such diets fed in abundant amounts may have less injurious effects upon subsequent growth capacity than does the feeding of restricted amounts of a balanced ration. Mendel ('14) has especially emphasized the difference between their experiments, in which the diet was ample in calories, and those of simple underfeeding with restricted amounts of a balanced diet. The relation of inanition to other factors producing abnormalities of growth is discussed by Mendel ('17).

It is also possible, however, that the very surprising results of Osborne and Mendel may be in part explained in another way. It is known that individual rats (and perhaps individual strains) may vary greatly in their resistance to inanition and in capacity for recuperation. In the experiments of Osborne and Mendel, it appears that some individuals are able to maintain their capacity for growth under conditions which would probably in most cases produce a permanent stunting or dwarfing of the body. To what extent this may be true in their experiments is uncertain, since they do not state the total number of animals under experiment, from which the successful cases were selected.

In our experience, as above stated, a large percentage of the animals die during the course of the severe underfeeding experiments, either directly from inanition or indirectly through lowered resistance to disease. Practically all of the survivors appear permanently stunted in their ultimate capacity for growth, although many of them upon autopsy reveal no evident disease. Accordingly, there seems no escape from the conclusion that while a considerable degree of inanition during growth may be followed by prompt and complete recovery upon ample refeeding, more severe and prolonged underfeeding, especially in the very young, reduces materially their capacity for subsequent growth and usually results in permanently dwarfed individuals.

BODY LENGTH AND TAIL LENGTH

The observations of Stewart ('18) indicate that at the end of the underfeeding period in young rats there is a relative elongation of the body (nose-anus) length, as compared with that in normal controls of the same body weight, amounting to an average of about 6 per cent in those underfed from birth to ten weeks, and about the same in those underfed for long periods beginning at three weeks of age. Jackson and Stewart ('19) have shown that in the case of the rats underfed from birth, the body length is still above normal on full refeeding to a body weight of 25 grams, but about normal in those refed to 50 or 75 grams. The present data (table 2) show the body length slightly subnormal (4.5 to 5.5 per cent) in such rats when refed for longer periods to maximum body weight. This means that in the test rats the body is relatively slightly plumper than in the controls. In the group refed after the longest period of underfeeding, however, as shown in the fourth column of table 2, there is practically no difference in body length between test rats and controls. It is therefore apparent that in any case no trace of emaciation remains in the underfed rats after refeeding to their maximum body weight.

The tail length in the rats underfed from birth increases even more than the body length (Stewart, '18), so the rats are relatively long-tailed at the end of the underfeeding period. On ample refeeding of such rats to a body weight of 25 to 75 grams, Jackson and Stewart ('19) find the ratio of tail length to body length nearly normal. The present data (table 2), however, indicates that in such rats, as well as those starved for longer periods, on amply refeeding to maximum body weight the tail becomes relatively somewhat short, averaging 4 to 8 per cent shorter than in the normal controls of the various groups. There is a slight sexual difference in tail length (that of the female being relatively slightly longer), but since the difference between test and control rats appeared nearly equal in each sex, the sexes are combined in the table.

TABLE 2

Average data for the parts, systems, and organs in the groups of albino rats fully refed to permanent size (adult) after underfeeding for various periods. Percentage differences (+ or −) between the data for test rats and for normal controls of similar body weight are indicated in parentheses. See note on the fourth group

	UNDERFED FROM BIRTH TO 3 WEEKS AND REFED	UNDERFED FROM BIRTH TO 10 WEEKS AND REFED	UNDERFED FROM 3 WEEKS TO 20 WEEKS AND REFED	UNDERFED FROM 3 WEEKS TO NEARLY 1 YEAR AND REFED
No. of test rats...	2 males, 3 fem.	1 male, 3 fem.	2 males, 1 fem.	7 males, 8 fem.
No. of controls...	4 males, 8 fem.	7 males, 9 fem.	5 males, 9 fem.	4 m, 8 f.[1]
Age of test rats...	445 da. (+18.0)	453 da. (+16.1)	340 da. (−12.2)	490 da.[1]
Body weight, g. ..	180 (−0.3)	200 (−2.0)	192 (+1.1)	129[1]
Body length, mm..	187 (−4.5)	190 (−5.5)	189 (−4.5)	176 (−0.2)
Tail length, mm...	159 (−8.0)	164 (−6.5)	163 (−5.9)	147 (−4.0)
Head, g..........	16.7 (−6.2)	18.5 (−1.6)	18.3 (−0)	14.6 (+6.6)
Fore limbs, g.....	11.7 (+4.5)	13.1 (+6.5)	11.9 (−1.7)	8.6 (+19.4)
Hind limbs, g.....	26.5 (−0.4)	29.8 (−1.9)	27.9 (−1.1)	19.7 (−1.5)
Trunk, g.........	121 (+1.7)	127 (−5.2)	128 (+1.6)	79.2 (−11.1)
Integument, g. ...	30.3 (−5.6)	33.7 (−7.4)	36.8 (+9.8)	22.5 (−7.8)
Skeleton, lig., g .	20.1 (−2.4)	19.3 (−11.9)	20.5 (−3.3)	16.1 (+5.2)
Skeleton, cart., g..	15.5 (−15.3)	14.6 (−24.7)	17.3 (−7.0)	14.1 (—)
Musculature, g...	71.8 (−4.1)	75.8 (−10.3)	83.9 (+5.8)	49.0 (−8.6)
Visceral group, g.	26.2 (+9.9)	26.0 (+3.6)	27.5 (+13.2)	20.1 (−3.8)
'Remainder,' g....	25.2 (+11.0)	33.5 (+26.9)	16.2 (−33.3)	14.4 (−11.7)
Brain, g..........	1.577 (−8.0)	1.679 (−4.2)	1.726 (−0.6)	1.690 (+2.8)
Spinal cord, g. ...	0.555 (−6.7)	0.605 (−1.3)	0.587 (−2.3)	0.514 (−0.9)
Eyeballs, g.......	0.319 (−2.1)	0.332 (+1.2)	0.292 (−10.7)	0.319 (+18.0)
Thyroid, g........	0.0297 (+23.2)	0.0240 (−5.5)	0.0234 (+13.1)	0.0153 (−13.2)
Thymus, g........	0.0409 (−29.9)	0.0542 (−12.2)	0.1029 (+85.7)	0.0439 (−7.8)
Hypophysis, m., g.	0.0077 (−7.2)	0.0081 (−10.0)	0.0092 (+8.2)	0.0058 (−15.3)
Hypophysis, f., g..	0.0112 (−2.6)	0.0101 (−18.5)	0.0094 (−24.2)	0.0068 (−5.7)
Pineal body, g.....	0.0018 (+20.0)	0.0015 (+7.1)	0.0009 (−40.0)	0.0012 (—)
Heart, g..........	0.852 (+8.7)	0.841 (+1.2)	0.934 (+14.9)	0.646 (+7.5)
Lungs, g..........	3.19 (−11.9)	2.87 (−14.3)	2.96 (−15.2)	4.94 (+31.4)
Liver, g...........	7.92 (+6.3)	8.27 (+3.4)	9.22 (+19.9)	5.09 (+8.5)
Spleen, g..........	0.637 (+11.8)	0.647 (+8.2)	0.516 (−12.6)	0.387 (−7.0)
Kidneys, g........	1.660 (+3.4)	1.623 (−7.4)	2.060 (+22.5)	1.186 (−0.9)
Stomach—intestines with contents, g........	13.83 (+15.1)	17.42 (+32.2)	14.41 (+14.0)	10.48 (—)
St.-Int., empty, g.	7.05 (+17.3)	8.42 (+27.6)	7.09 (+12.7)	4.41 (−3.3)
Suprarenals, m., g.	0.0374 (−17.6)	0.0370 (−11.1)	0.0479 (+10.6)	0.0362 (+4.5)
Suprarenals, f., g..	0.0499 (+1.5)	0.0563 (+12.6)	0.0396 (−20.8)	0.0400 (+17.8)
Ovaries, g.........	0.0451 (−35.5)	0.0306 (−56.8)	0.0482 (−32.0)	0.0355 (−61.5)
Testes, g..........	1.805 (+8.7)	2.232 (+17.6)	2.307 (+30.3)	1.667 (+17.1)
Epididymides, g...	0.613 (+22.8)	0.745 (+32.6)	0.637 (+24.4)	0.512 (+34.0)

[1] In the fourth group, comparisons between test rats and controls for the organs were made through the Wistar norms by Donaldson's method. For the body parts and systems, the normal was calculated from the data of Jackson and Lowrey, as explained in the text.

HEAD, LIMBS AND TRUNK

In rats underfed from birth to three weeks of age, Stewart ('18) found the head about 16 per cent above normal weight, but in those underfed to six or ten weeks the head was nearly normal. In such underfed rats, on ample refeeding to a body weight of 25 to 75 grams, Jackson and Stewart ('19) found the head weight nearly normal. Similarly in the present series (table 2), refed to maximum body weight, the head weight is nearly normal, the relatively small differences being probably of no significance.

The limbs and trunk show no marked or constant change in relative weight during the underfeeding period in young rats (Stewart, '18), and likewise during ample refeeding up to body weights of 25 to 75 grams (Jackson and Stewart,'19). Similarly in the present series, the limbs and trunk in general show no marked deviation from the normal weight, except in the last group (table 2, fourth column). Even in this case, the excess weight of the fore limbs (+19.4 per cent) and the subnormal weight of the trunk (−11.1 per cent) are of questionable significance. The limbs are difficult to separate from the trunk in a uniform way, and, as previously explained, no direct controls were available for this group. On the whole, therefore, it is doubtful whether the refed test rats show any significant abnormality in the proportions of their head, limbs, or trunk.

THE BODY SYSTEMS

These body systems include the integument, skeleton, musculature, visceral group, and 'remainder.'

Integument. In rats underfed from birth, Stewart ('16) found the integument nearly normal in weight at three weeks, but 43 to 48 per cent subnormal at six and ten weeks of age. On refeeding such rats, Jackson and Stewart found the integument still subnormal at a body weight of 25 grams, but normal (or above) at 50 and 75 grams. The present series (table 2) shows the integument averaging slightly subnormal (−5.6 to −7.8 per cent) in all, but the third group (+9.8 per cent). The differences are of questionable significance, as the skin is quite variable in

weight, but it would seem that the relative plumpness of the body (above referred to) is not due to accumulation of subcutaneous fat.

Skeleton. The 'ligamentous skeleton' includes the bones, cartilages, periosteum, and ligaments. The fibrous structures were removed by maceration (see earlier papers for technique), leaving the 'cartilaginous skeleton,' which was oven-dried to constant weight as the 'dry skeleton.'

A remarkably persistent overgrowth of the skeleton in general has been observed during underfeeding in calves (Waters, '08), puppies (Aron, '11) and rats (Jackson, '15). Increase in body length, indicating continued increase in skeletal growth, with retardation in body weight, has also been noted in malnourished human infants and children (Variot, '07; Freund, '09; Birk, '11; Hess, '16). In rats underfed from birth, Stewart ('18) found at three to ten weeks an excess weight of 19 to 24 per cent in the ligamentous skeleton, 33 to 94 per cent in the cartilaginous skeleton, and 46 to 166 per cent in the dry skeleton. In such rats, on ample refeeding to 25 to 75 grams in body weight, Jackson and Stewart ('19) found that in general the skeletal weight has dropped to normal proportions, or even subnormal (cartilaginous and dry skeleton), suggesting that although skeletal growth persists during early inanition periods, its later growth may be inhibited. The present data (table 2) tend to confirm this in the test rats amply refed to maximum body weight. While the condition in the last group is more uncertain (due to lack of direct controls, as previously explained) the other groups show uniformly subnormal skeletal weight. While small in the ligamentous skeleton (−2.4 to −11.9 per cent), it is more definite in the cartilaginous skeleton (−7.0 to 24.7− per cent), which probably accounts for the slightly subnormal body length. A few observations on the weight of the dry skeleton (not given in the table) show a slight deficit in the test rats, but they are too few to be conclusive.

Musculature. In rats underfed from birth to three, six, or ten weeks of age, Stewart ('18) found the musculature slightly (8 to 12 per cent) above normal weight. In such rats, on ample

refeeding to a body weight of 25 to 75 grams, Jackson and Stewart ('19) found no excess, but rather a slight deficit (−5.7 to −15.4 per cent) in the musculature of the rats underfed to six or ten weeks. This indication that early inanition may exert a retarding effect on the later growth of the musculature is to a certain extent supported by the present data for such rats refed to maximum body weight. As shown in table 2, there is a slight underweight (−4.1 to −10.3 per cent) in all except the third group, which instead averages 5.8 per cent above normal. These differences are so slight that their significance is somewhat doubtful, though a negative tendency is apparent.

Visceral group and remainder. The visceral group, which includes all the individual organs listed in table 2, is found 28 to 38 per cent above normal weight in young rats underfed from birth to three, six, or ten weeks of age (Stewart, '18). Jackson and Stewart ('19) found the group as a whole nearly normal in weight when such rats were amply refed to a body weight of 25 to 75 grams. The present data (table 2) show the visceral group slightly above normal weight (+3.6 to +13.2 per cent) in all but the last column (−3.8 per cent). There are differences in the individual organs, however, as will be discussed later.

The 'remainder' is obtained by deducting from the net body weight (intestinal contents excluded) the weight of the integument, skeleton, musculature, and visceral group. This 'remainder' includes various small, unweighed organs, dissectible fat, body fluids, loss by evaporation, etc. In rats underfed from birth to three, six, or ten weeks, Stewart ('18) found a marked loss (−19 to −40 per cent) in the weight of the 'remainder.' No constant or significant variations from the normal occurred in such rats amply refed to a body weight of 25 to 75 grams (Jackson and Stewart, '19). In the present series, the 'remainder' is considerably above normal in the refed rats which had been underfed from birth, but below normal weight in those underfed beginning at three weeks of age (table 2). The interpretation is doubtful, as the 'remainder' is exceedingly variable even under normal conditions.

The individual viscera. In rats underfed from birth to three, six, or ten weeks, Stewart ('18) found in general a marked relative increase in the weights of the spinal cord, eyeballs, liver, stomach, and intestines (empty). A less marked tendency to increase occurred in the brain (especially in the earliest period), heart (progressive increase), spleen (at six or ten weeks), intestinal contents (at six or ten weeks), suprarenals (progressive increase), kidneys, testes, epididymides (at three and six weeks, loss later) ovaries and hypophysis. No marked change was found in the weights of the thyroid and pineal gland, but a definite loss of weight occurred in the lungs and especially in the thymus. In the earliest period only (up to three weeks) there was a loss in the weights of spleen and intestinal contents.

In rats underfed for very long periods (beginning at three weeks of age), Stewart found the earlier well-marked overweight still evident in the spinal cord, eyeballs, and (usually) the suprarenals. The brain showed a slight increase (above normal), and also the lungs (probably abnormal). There was apparently no marked change (or inconstant variability) in the weights of the intestinal contents, kidneys, ovaries, testes (?), and pineal body. The thyroid, thymus, heart, liver (variable), spleen (variable), alimentary canal (empty), epididymides and hypophysis (male) usually became subnormal in weight during the long underfeeding periods.

In rats underfed from birth to three, six, or ten weeks and amply refed to a body weight of 25 to 75 grams, Jackson and Stewart ('19) found considerable variation in the extent of recovery in weight among the individual viscera. The hypophysis, suprarenals, heart, lungs, and kidneys were usually normal, the liver irregular in weight. The brain, spinal cord, and thymus were almost constantly subnormal, pineal, thyroid, and epididymides somewhat irregular. The ovaries were also irregular, apparently subnormal in rats refed after underfeeding to ten weeks of age. The stomach and intestines and the eyeballs were usually somewhat above normal weight. The spleen and the testes showed an apparent overweight in the earlier stages, with a subnormal tendency later.

In the present series (table 2) the individual organs in the various groups of underfed rats refed to maximum body weight may be mentioned briefly.

The *brain*, as seen in table 2, remains subnormal (−4.2 to −8.0 per cent) in the rats refed to maximum weight after underfeeding from birth, although somewhat nearer normal than in those refed to body weight of 25 to 75 grams (previously mentioned). Thus underfeeding at this early period, while not preventing a marked temporary overgrowth of the brain (with normally proportioned parts, according to Stewart, '18 a), apparently exerts an inhibitory influence on the later growth of the brain, which is retarded so as to lag slightly behind the body as a whole in its ultimate growth in weight.

On the other hand, the longer underfeeding period beginning at the later age of three weeks is apparently less disturbing in its effects upon the growth of the brain, which is nearly normal in weight (relative to the body) both at the end of the fasting period and at the end of the refeeding period (table 2, last two columns). Hatai ('07) likewise found a normal weight for the central nervous system of rats refed after partial starvation (starch diet) for three weeks in rats one month old. King ('16), however, found both brain and spinal cord subnormal in weight in dwarfed rats which were undersized ('runts') from birth. As suggested above, this condition may possibly be due to prenatal malnutrition.

The *spinal cord*, though subnormal in weight in the rats refed to body weight of 25 to 75 grams, after underfeeding from birth (Jackson and Stewart, '19), is more nearly normal in the present series refed to maximum weight. As seen in table 2, the first group (−6.7 per cent) is the only one showing any appreciable deviation from the normal.

The *eyeballs* which grow to a remarkable extent during early inanition and are still somewhat above normal weight on refeeding to a body weight of 25 to 75 grams, are nearly normal in corresponding rats refed to maximum body weight (table 2, first two columns). Of those underfed at a later period, one group shows an underweight (−10.7 per cent), the other an overweight (+18.0 per cent) in the eyeballs, the difference being of uncertain significance.

The *thyroid gland*, which was found somewhat variable in its weight at the end of the inanition periods and on refeeding to a body weight of 25 to 75 grams, appears likewise somewhat inconstant in the present series (table 2) refed to maximum body weight. The results do not warrant any more definite conclusion.

The *thymus*, which undergoes a marked loss of weight during 'hunger involution,' was found still subnormal upon refeeding to a body weight of 25 to 75 grams (Jackson and Stewart, '19). The subnormal weight still persists in three groups of the present series, refed to maximum body weight (table 2). In one of the groups, however, there is a remarkable overweight of 85.7 per cent, probably due to experimental error in removing fat along with the thymus.

The *hypophysis* has been found by Hatai to show a sexual difference in weight, therefore the data for this gland are listed separately by sex in table 2. In most cases (excepting in the male on long underfeeding) it was found nearly normal at the end of the underfeeding periods, and on refeeding to a body weight of 25 to 75 grams. In the present series, refed to maximum body weight, it is more or less subnormal in all but one group (table 2), which shows a slight overweight (+8.2 per cent). Jackson ('17) found that in young rats refed four weeks or more after inanition the hypophysis has for the most part recovered its normal histological structure, although atrophic areas may persist for indefinite periods.

The *pineal body* was found irregular in weight in the underfed rats on refeeding to body weight of 25 to 75 grams. Likewise in the present series (table 2), it shows an apparent overweight in the first two groups, but a decidedly subnormal weight in the third. On account of the small size of the gland, no definite conclusion can be drawn from the few observations.

The *heart* shows a tendency to increase in weight in the earlier underfeeding periods, but loses weight later. It was usually found nearly normal in the rats refed to a body weight of 25 to 75 grams. In the present series, refed to maximum body weight, the heart shows a tendency to hypertrophy, which is distinct in

three of the four groups (table 2), the overweight varying from 1.2 to 14.9 per cent.

The *lungs*, which were nearly normal in weight in the underfed rats refed to body weight of 25 to 75 grams, are distinctly subnormal in three of the four groups of the present series (table 2). The enormous overweight (+314 per cent) in the fourth group is undoubtedly pathological, due to a chronic lung disease, which occurs frequently in the older rats.

The *liver*, which shows a marked growth tendency during the earlier inanition periods, is variable in weight later, and in the rats refed to a body weight of 25 to 75 grams. In the present series (table 2) refed to a maximum body weight, the liver appears above normal in all groups (+3.4 to +19.9 per cent). On account of the great normal variability in the size of the liver, however, the significance of this apparent hypertrophy is somewhat uncertain.

The *spleen* is exceedingly variable in weight, both normally and after various inanition periods. In the rats refed to body weight of 25 to 75 grams, the spleen was found usually above normal in the earlier stages and subnormal later. In the present series refed to maximum body weight (table 2), the spleen appears somewhat above normal (+8.2 and +11.8 per cent) in the groups underfed from birth, but below normal (−7.0 and −12.6 per cent) in those underfed beginning at three weeks of age. It is uncertain as to how much of this difference is due merely to normal variability.

The *kidneys* were usually found normal in weight in the rats refed to a body weight of 25 to 75 grams. In the present series, refed to maximum body weight (table 2), they likewise differ but little from the normal, excepting the third group, which appears above normal (+22.5 per cent). As the kidneys normally show considerable variation in weight, this exception is of doubtful significance.

The *stomach and intestines* (without contents) show a remarkably strong tendency to increase in weight during the earlier underfeeding periods, though becoming subnormal during the later and longer fasting experiments. On refeeding (after early

underfeeding) to a body weight of 25 to 75 grams, the empty stomach and intestines still appeared somewhat above normal in weight. In the present series refed to maximum body weight, an over-weight persists in the alimentary canal, both with and without contents, excepting in the fourth group (table 2). A period of inanition in the young, growing rat therefore apparently tends to produce a permanently hypertrophied condition in the alimentary canal (stomach and intestines).

The *suprarenal glands*, like the hypophysis, show a sexual difference in weight (Hatai, Jackson). They usually increase above normal weight in young rats underfed for various periods, but were found nearly normal on refeeding to a body weight of 25 to 75 grams. In the present series refed to maximum body weight, they appear irregular (table 2), some groups being above normal, others subnormal in weight. As the suprarenals are normally somewhat variable and the number of observations rather small, no definite conclusion is justified. The histological changes in the suprarenal glands during inanition and refeeding have been described by Jackson ('19).

The *ovaries* appear especially affected by inanition during the early growth period. As shown by Stewart ('18), they increase markedly in weight in rats underfed from birth to three, six, or ten weeks, but are somewhat variable in those underfed for long periods beginning at three weeks of age. In the former group, Jackson and Stewart ('19), in rats refed to a body weight of 25 to 75 grams, found the ovaries irregular in weight, usually subnormal in those which had been underfed to ten weeks.

The present data (table 2) indicate that in the rats underfed beginning either at birth or at three weeks of age, the later growth of the ovaries is profoundly affected. They average from 32 to 61 per cent below normal in the rats refed to maximum body weight. This subnormal weight of the ovaries indicates an atrophy which is doubtless sufficient to account for the marked loss of reproductive power in the test females. This was discussed earlier in the paper. Since these females were mated with normal males (the controls), their failure to reproduce cannot be ascribed to the males.

The more favorable results by Osborne, Mendel and Ferry ('17), who obtained three to five litters in four of their female rats refed after stunting by inadequate diet with retarded growth, is perhaps due to the fact that their inanition began at a later age (five or six weeks) and terminated with a body weight (90 to 108 grams) nearly double that in our rats underfed for the longest period. It is quite possible that there may be a critical period in the development of the rat's ovary, before the age of five or six weeks, when inanition is much more injurious than later.

The *testes* react somewhat like the ovaries (in weight) during the earlier inanition periods, but the ultimate effect is different. Stewart ('18) found the testes increased in weight in rats underfed from birth to three, six, or ten weeks, but variable or decreased in those underfed for longer periods beginning at three weeks of age. In the rats refed to body weight of 25 to 75 grams after underfeeding from birth, Jackson and Stewart ('19) found an apparent overweight in the testes in the earlier periods of refeeding, but a subnormal tendency later.

The present data (table 2) for the underfed rats fully refed to maximum body weight show a surprising tendency to overweight in the testes, the average varying from about 9 to 30 per cent in the various groups. Since these test males were not separately mated with normal females, it is uncertain whether their normal reproductive function was restored.

The *epididymides* in general during inanition in young rats undergo changes in weight somewhat similar to those in the testes, tending to increase in weight in early stages, with loss in weight later (Stewart). They were found irregular in weight in rats refed to a body weight of 25 to 75 grams. In the present series (table 2) in the rats refed to maximum body weight, the epididymides show a hypertrophy even greater than that of the testes, the overweight being from 23 to 34 per cent in the various groups.

SUMMARY

1. Albino rats fully refed after underfeeding from birth to three, six, or ten weeks, or from three weeks to nearly a year of age, grow variably, but usually fail to reach the normal adult size. The ultimate effect varies according to the length of the underfeeding period, the age at which the inanition occurred, the sex (body weight more affected in males), the severity and the character of the inanition.

2. In the test rats refed to maximum body weight, the body length and tail length appear slightly subnormal, head, limbs, and trunk nearly normal in weight; skeleton, integument, and musculature usually slightly subnormal, visceral group slightly above normal, and 'remainder' variable.

3. Of the individual organs, the brain, spinal cord, hypophysis, and lungs of the test rats average slightly subnormal in weight, the ovaries very markedly so. The atrophy of the ovaries probably accounts for the reduction of reproductive capacity in the test females, which is especially marked after the long underfeeding periods. The heart and alimentary tract appear slightly, and the testes and epididymides very definitely, above normal weight. The other organs appear either normal or irregular in weight, in comparison with normal controls of the same body weight.

4. While some abnormalities thus occur in the test rats, they are usually slight, and in general it may be said that the organs and parts are nearly normally proportioned in the permanently stunted rats. Thus the earlier starvation apparently retards or inhibits the later growth process of the body as a whole, with the few exceptions above noted.

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Resumen por el autor, J. A. Dawson.
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Estudio experimenral de un Oxytricha amicronucleado.

II. La formación de animales dobles o "gemelos."

En los cultivos de Oxytricha hymenostoma, una especie amicronucleada, bajo condiciones semejantes a aquellas en las que tiene lugar la singamia en las formas hipótricas, hay una marcada tendencia a la formación de animales dobles o "gemelos" por fusión plastogámica dorsal. Los gemelos se reproducen por fisión binaria, cuyo resultado general es la producción de dos pares de gemelos en un todo semejantes a los padres. Tales gemelos han aparecido en varios cultivos, por ejemplo, en los procedentes de troncos normales, en los descendientes de animales sencillos procedentes de gemelos, y en la progenie de los animales caníbales. Para la existencia continua de formas bemelas son necesarias condiciones ambientes favorables. Los experimentos de selección indican la posibilidad de obtener troncos gemelos que se reproducen fieles a su tipo de un modo indefinido. La cantidad de divisiones de los gemelos en un tiempo determinado es semejante a la de los animales sencillos normales. La condición miscible del citoplasma que conduce a la formación de gemelos continúa mientras vive el animal. Cuando se forman gemelos y se mantienen las condiciones ambientes favorables, la fusión es generalmente permanente, es decir, hay posibilidad de que las formas gemelas vivan indefinidamente.

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AN EXPERIMENTAL STUDY OF AN AMICRONUCLEATE OXYTRICHA

II. THE FORMATION OF DOUBLE ANIMALS OR 'TWINS'[1]

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TWENTY-TWO FIGURES

CONTENTS

1. INTRODUCTION

The occurrence of double animals, or as they are usually termed, double monsters, is not uncommon among the Infusoria. Although they most frequently occur during experiments where merotomy has been practiced, yet the 'spontaneous' occurrence of such forms has been noted several times.

The first observations of importance in respect to formation of double animals were made by Balbiani ('91), working with Stentor, who described two monsters, one of which was formed from a posterior 'merozoite' which was again cut longitudinally in the posterior end. Traces of duality were seen after the first

[1] It is realized that there are certain objections involved in the use of the word twin as applied here, but inasmuch as there is apparently no single English word which is more suitable, this term is used at present to designate the double animal.

division, but these gradually disappeared, leaving two apparently normal animals. A second monster occurred 'spontaneously,' but in this specimen the double characters were limited chiefly to the peristomes. The same author ('93) obtained by merotomy a series of multiple monsters which arose from the double monsters which are invariably produced by the failure of a 'merozoite' to complete the process of fission. Johnson ('93) described a double monster in Stentor where the duality was limited to the anterior part of the body. All traces of duality disappeared in a few days. Simpson ('01) observed a double Paramecium which had arisen by the failure of an exconjugant to complete fission. Here the two bodies formed one continuous animal which gave off daughter cells from each end for three generations, after which it developed further abnormalities of form and later died. Prowazek ('04) obtained double monsters from fragments of Stentor. The same author ('04 a) observed in degenerating cultures of Stylonychia a certain amount of hyperregeneration. He figured an individual which had two posterior ends while the mouth and cirri had suffered marked reduction. Jennings ('08) described various abnormalities occurring in cultures of Paramecium. In particular he obtained a race with a marked tendency to form chains, due to the failure of individuals to complete the process of fission. Occasionally a double individual pulled apart, producing paramecia with posterior spines which were handed on to the posterior daughter cells for a few generations. Mast ('09) described double and triple monsters in Didinium which apparently consisted of individuals fused posteriorly. No explanation was given of the origin of these monsters, although the author pointed out that they obviously had not arisen from a failure to complete fission, since the posterior ends were always in contact. In only one case was an attempt at reproduction noted. Calkins ('11) produced double and multiple monsters by cutting paramecia. Vitality was weak in these abnormal forms and death usually occurred in a short time. Peebles ('12) obtained, by cutting, fragments of paramecia which did not complete fission and thus formed 'chain monsters.' Stocking ('15), working on the in-

heritance of abnormalities occurring after conjugation in Paramecium caudatum, described many types of constantly recurring abnormalities, among others were noted certain types of double forms, arising on account of 'arrests in development,' which invariably died in a short time. The same author described a certain class of conjugants which never separated but died while united.

The preceding account deals with double or multiple forms arising from one individual. Cases of long-continued plastogamy in the ciliates are rare and are limited to fragmentary observations on compound organisms formed when three or more individuals attempt to conjugate. Engelman stated that these individuals could grow and multiply, while Maupas believed such fusions had nothing to do with conjugation, but resulted in monstrous formations which underwent incomplete and irregular divisions. Doflein ('07) described 'agamic fusions' of two individuals in Paramecium putrinum and Stylonychia mytilus, in which cytoplasm and nuclei of each animal fused to give an apparently normal but relatively very large individual. Doflein ('09) states further that as a rule plastogamic individuals did not long remain united, but separated after a longer or shorter time.

2. MATERIAL AND METHODS

The ancestors of all animals described in these experiments were descendants of stock animals of the culture (A) of a race of Oxytricha hymenostoma. (See 'An Experimental Study of an Amicronucleate Oxytricha,' part I).

A full description is given in part I of the methods used in carrying on the daily isolation cultures, the preparation of the culture medium, the method of plotting the curves, etc. As the methods used in this part of the study are the same, the reader is referred to the previous paper for these details.

3. THE OCCURRENCE AND BEHAVIOR OF DOUBLE ANIMALS

During the progress of the previous work (part I), while studying a six-day-old mass culture made on July 20, 1917, from a stock slide of culture A, numerous pairs were observed, while many individuals were still dividing by fission. In addition to the paired animals which were apparently attempting to conjugate (pp. 482, 483, part I), three pairs of animals were found

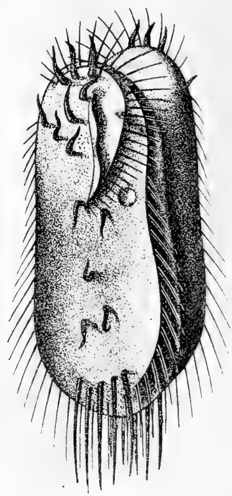

Fig. 1 Normal twin drawn from life, magnified about 700 diameters, showing ventral surface of one animal and a portion of dorsal (aboral) surface of the other.

evidently fused in what appeared to be a very unusual manner. These were isolated and the next morning were found to have divided twice, giving in one case four double animals exactly similar to the parent double animal. These animals thus isolated formed the ancestors of the four lines of a culture (AT) which was carried with the object of studying later developments in the life-history of these 'abnormal' forms and for the purpose of comparing their reproduction with that of the animals in cul-

ture A. Subsequently the formation of double animals has occurred repeatedly in other cultures of this amicronucleate race, but all such forms coming directly from culture AT are designated as Twin Series 1 (p. 136).

A thorough study was made of some of these double individuals, which showed that they could be interpreted as two single

Figs. 2 to 5 Photographs of a model constructed from drawings of a typical double animal. Figure 2, ventrolateral view; figure 3, ventral view; figure 4, lateral view; figure 5, end view (anterior).

animals fused together along the middorsal line. As will be seen from the following text figures, the twins were slightly separated anteriorly, but were firmly united along the dorsal side to the posterior end. Figure 1 shows the usual appearance of the living twin.

The behavior of the normal single animal is similar to that described by Jennings ('06) for Oxytricha. On account of the double character of the twin, its behavior is somewhat different from that of the single animal. During creeping, the cirri of only one of the components are in contact with the substratum and the creeping movements are not active as those of the single animal. In free-swimming, however, the twin has a characteristic rapid rotation on its long axis which is in striking contrast to the slow rotation of the single animal.

Reproduction of the twin form takes place by transverse fission, just as in the single animal, with the notable difference that, whereas the daughter single individuals are constricted off sharply at the point of separation, the daughter twins usually remain connected for some minutes by a thin strand of protoplasm which gradually pulls out into a finer and finer thread and is finally broken. The unusual spectacle is thus presented of the anterior twin swimming actively and rotating rapidly while at the same time drawing after it the posterior cell in which the rotation is not nearly so marked. The connecting strand, which is invisible except under a magnification of about 600 diameters, occasionally reaches a length fully three times that of the daughter twins. The peculiar physical condition of the protoplasm, shown by the tendency for individuals to remain connected for a longer or shorter time by cytoplasmic strands, is highly significant, since it indicates the continuance of the 'miscible' state of protoplasm which made possible the formation of twin animals. The phenomenon just described has been noted throughout the life of the twin race, which, in this series, lasted for over one hundred generations.

After twins had been under observation for a short time, it was found that single animals sometimes were given off during reproduction by fission. This production of single animals occurred most frequently in the following manner. A slightly more marked separation of the anterior ends of the individuals forming the twins (as shown in fig. 17) gave, when fission occurred (fig. 18), a normal twin connected only in the posterior region. Once separation in this way had begun, it invariably resulted in the complete pulling apart of the twin animals.

As the separation became more marked the ventral surfaces of both animals rested on the bottom, as shown in figure 19. The ensuing tug-of-war caused the separation of the twin, usually in half an hour to an hour. During this process there always occurred a pulling out of the protoplasm into a strand of varying diameter, but never so fine as that observed in the reproduction of a twin by fission. Figure 20 shows a twin in which such a connecting strand is present. After the break occurred, the remains of the strand could be seen for some time on each individual as a posterior dorsal spine (fig. 21) entirely comparable to that described by Jennings ('08, p. 625). This spine, however, is invariably completely resorbed within an hour or so and has never in the history of any of the strains been transmitted at fission to a daughter animal.

When the amount of anterior separation of a twin was somewhat greater than in the case just described, the fission which followed resulted in the direct separation of two single animals from the separated anterior ends of the present twin, while the posterior portion remained a twin animal. In rare cases a twin, apparently normal, would pull apart before fission occurred, thus giving two single animals. In carrying pedigrees of twin animals, single animals which had arisen in this way are always regarded as of the same generation as the twin from which they came.

The methods of multiplication of twins may be summarized as follows:

1. A twin divides and thus gives rise to two typical twins (figs. 15 and 16).

2. A twin, somewhat separated anteriorly, divides, producing, from the anterior end, a twin widely separated anteriorly—and from the posterior end, a typical twin. The widely separated daughter twin pulls apart to give two single animals, typical in every respect except for the dorsal spines which are invariably soon resorbed and are never transmitted to the next generation (figs. 17, 18, 19, 20, and 21).

3. A twin with a marked separation of the components at the anterior end divides, resulting in two single typical animals from the anterior end and a twin, usually with only slight anterior separation, from the posterior end.

4. A twin at first apparently fused in the typical manner pulls apart to give two single animals normal except for dorsal spines.

It is noteworthy that the miscible condition is soon lost by the single animals derived from twins, since it has been observed invariably that such animals separate during fission in the same manner as the single animal not of twin origin; i.e., there is an almost direct severing of the daughter cells with no pulling out of a protoplasmic strand. Since in the case of both twins and single animals derived from twins the environment was the same, it is clear that the environment, though probably the chief factor in bringing about the miscible condition of the protoplasm necessary for the formation of the twin animal, is not responsible for the continuance of this condition. In other words, the 'miscible' condition of the protoplasm persists as long as the twin strain persists, and disappears as soon as the components of the twin separate.

The complete dual nature of the double animal or twin cannot be too strongly emphasized. All of the external structures found in single animals have been duplicated in each component of a twin except in the region of fusion. This duality was not limited to external features alone, but it has been found almost invariably that the internal structures of each component were not visibly affected by the union. In a few exceptional cases, however, a certain amount of macronuclear fusion has been observed (fig. 22). Animals in this condition do not survive long and there is no evidence to show that such fusion plays any significant part in the life-history of the twin animal. As in the single animals, here also a certain amount of fragmentation of the macronuclei has been observed (figs. 17, 18, and 20). The usual condition of the macronuclei is shown in figures 15 and 16.

4. EXPERIMENTS ON PEDIGREED 'TWIN' CULTURES

a. History of culture AT (series I)

On July 28, 1917, from the four progeny of one of the twins isolated on the previous day, the four lines of the main culture of twins (AT) were begun. This twin culture was carried by

daily isolations from July 28th to November 18th, and finally died in the one hundred and second generation, outliving the 'normal' culture (A) by several days. The graph showing the average division rate of the four lines for five-day periods has been plotted together with that of culture A.

Figure 6 shows at a glance absolute and relative division rates of the parent culture (A) and its twin derivative (AT). It is

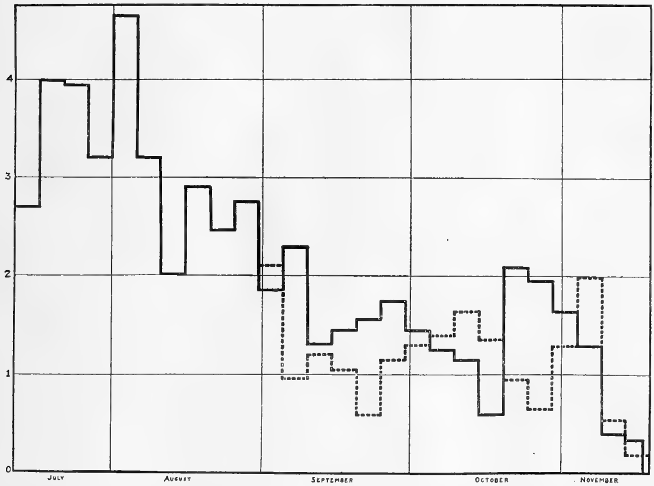

Fig. 6 Comparison of normal culture, A (continuous line), and twin culture, AT (broken line). Culture AT begins July 28, 1917. Culture A died out on November 17, 1917. The death of culture AT occurred during the next five day period (not shown in figure).

apparent that the average division rates of A (after AT arose, July 27, 1917) and AT are essentially the same; the various fluctuations might well represent those of two cultures of normal animals from the same ancestor. The twin culture outlived the culture of 'normal' animals for a few days, and finally the last representative of culture AT died in the one-hundred-and-second generation. During the last week of the life of the twin culture

the animals did not divide by fission, but became smaller, darker in appearance, and more sluggish in movement. Specimens stained during this period had a normal structure when examined cytologically, the decrease in size being the only noteworthy feature.

Attempts have been made to keep these twins alive by means of mass culture similar to those used to keep alive representatives of culture A, but it has invariably been found that they soon disappear from such cultures—the maximum time before their total disappearance being three weeks—although the single animals derived from the twins multiply and live as long as conditions are kept favorable.

The elimination of twins in mass cultures is probably due to the combined operation of several factors. In the course of fission a twin frequently divides giving one twin and two single animals. In a few generations it will be readily seen that twin animals tend to be outnumbered by single animals. The only instance in which a twin shows greater activity than a single animal is in its rapid rotation while swimming. At all other times the single animal moves more actively, which undoubtedly aids in its securing a better food supply. Possibly, owing to the fact that the single animal has a greater surface per unit volume, it is better able to carry on metabolic processes. The survival of single animals recalls the observation by Jennings ('08) in which he found that united specimens of paramecia in mass cultures soon disappeared. This he attributed to their more sluggish movements and consequent inability to obtain an adequate food supply in competition with the more active single animals.

b. Effect of selection on 'twins' (series I)

When the original twin the progeny of which formed culture AT was isolated, a series of experiments was begun using animals from the twin culture (AT) in order to find out if a strain could be obtained which would breed true for twins. Cultures were carried of animals which gave 100 per cent of twins for three or four generations. As the number of such cultures which could be

carried was obviously limited, it was decided to carry three at a time, isolating the animals as soon as fission occurred and keeping all progeny for six or seven generations. Selection was then made of the most typical twins in these generations. After three generations the twin which had divided to give the eight animals showing most promise of breeding true was used as the starting-point of the succeeding culture. Thus a continued process of selection was used, and the result indicates that a strain of twins

TABLE 1

A summary of a series of cultures carried in the experiment to determine the effect of artificial selection in twin lines taken originally from animals of series I (culture AT). The continuity of the various subcultures is shown in columns 1 and 3 of the table. The percentage of animals remaining twins throughout the series is shown graphically in figure 7.

CULTURE NUMBER	NUMBER OF GENERATIONS IN WHICH ALL ANIMALS IN CULTURE (COL. 1) WERE ISOLATED	FROM CULTURE	NUMBER OF GENERATION FROM WHICH SUCCEEDING CULTURE WAS BEGUN	PERCENTAGE OF ANIMALS REMAINING 'TWINS' IN GENERATION NUMBER						
				1	2	3	4	5	6	7
T 1	7	AT, 5th gen.	7	100	100	100	68.4	56.8	40.3	33.3
T 4	8	T 1	6	100	100	100	77.7	64.1	51.9	44.2
T 8	7	T 4	6	100	100	100	77.7	51.1	47.6	43.7
T 15	8	T 8	6	100	100	100	68.4	48.4	27.3	16.7
T 17	7	T 15	10	100	100	100	100	68.4	52.4	36.2
T 24	7	T 17	6	100	100	100	68.4	56.1	47.1	32.6
T 25	8	T 24	7	100	100	100	68.4	65.8	59	47.5
T 35	7	T 25	7	100	100	100	100	92.2	71.4	56.2
T 37	8	T 35	10	100	100	100	100	93.9	77.8	66.3
T 41	8	T 37	7	100	100	100	100	87.5	83	69.0
T 44	7	T 41	11	100	100	100	100	100	100	96.3
T 47	8	T 44		100	100	100	100	100	88.7	84.0

was obtained which showed less tendency to separate, and toward the end of the life of series I, one culture was obtained which through the seventh generation produced 100 per cent twins.

A study of table 1 shows further that during the progress of this selection the tendency to remain united became gradually more and more marked. Unfortunately, when a strain of twins was thus obtained which gave every promise of continuing to breed true the whole series died out. It is believed that the culture medium was largely responsible for the death of the series,

since stock animals from culture AT which had been carried from previous cultures of the same race continued to thrive in the Petri-dish cultures and representatives of the race are still living,

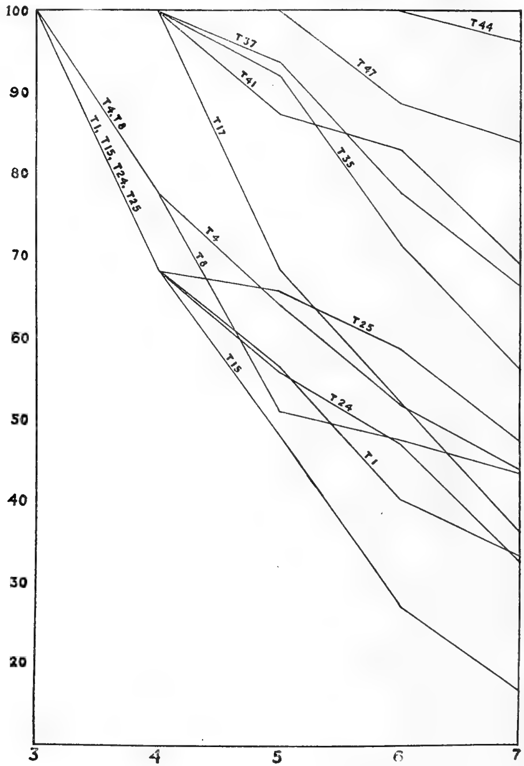

Fig. 7 The ordinates represent the percentage of animals remaining twins. The abscissas represent the generation number. All cultures remained 100 per cent twins for the first three generations.

although no twins are present. It is noteworthy that in stock cultures the twins, which were bred late in the history of culture AT, remained twins longer than was the case when their occurrence was first noted.

5. STUDY OF THE ORIGIN OF 'TWINS'

After the initial occurrence of twins, a series of mass cultures of single animals was carried both in Petri-dish cultures and on depression slides for the purpose of ascertaining if further twin formation would occur. These cultures consisted of stock ani-

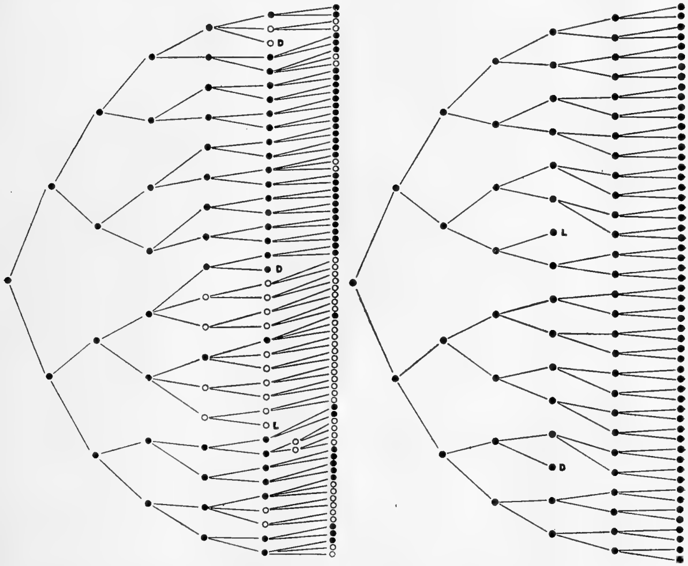

Fig. 8 Comparison of typical pedigrees of early selection series (A) and series after considerable selection (B). A represents pedigree of subculture T4 (fig. 7 and table 3). Full black circles represent twins; circles represent single animals derived from twins. *D*, animal died. *L*, accidentally lost. In the fifth generation (A) an example occurred where a twin (apparently fused normally) separated completely giving two single animals, each of which is therefore still in the fifth generation.

mals from culture A in which twin formation had not occurred during the course of the experiment, of stock single animals from the various subcultures of series I, i.e. consisting of typical single animals descended from twin ancestors and of stock animals coming from cannibal ancestors.

During the experiment, twin formation has occurred among animals from each of these sources. In all, eight different series have been obtained. The manner of formation and history of each series follows.

Series II. The ancestor of series II was a twin isolated as soon as its occurrence was noted, from a thirty-day-old mass culture in a Petri dish of single animals from culture T 15 (see Selection Experiment) on December 16, 1917. The progeny of this animal comprising culture S2T were isolated as soon as fission occurred in each case up to the fifth generation. Following is a record of the culture:

DATE	GENERATION	'TWINS'	SINGLE	PER CENT REMAINING TWINS
12–17	1st	2		100.0
12–18	2d	3	2	60.0
12–19	3d	6	4	60.0 (1 twin died)
12–20	4th	9	10	47.7
12–21	5th	15	26	36.3 (3 twins died)

One of the twins from the fifth generation divided to give eight twins, all of which were isolated to form subculture S2T1.[2] Of the eight, three died, and the remaining animals in the fourth generation produced eight twins and four single animals (66.7 per cent remaining twins). The fifth generation gave fourteen twins, twelve single animals (53.8 per cent). The most vigorous animals were carried along by daily isolations, and, from descendants of these, several subcultures of four lines each were started.

The subsequent history of these subcultures is summarized as follows:

NUMBER OF SUBCULTURE	FROM CULTURE	GENERATION IN WHICH SUBCULTURE WAS BEGUN	GEN. REACHED BY SUBCULTURE	TOTAL GEN. FOR SERIES
S2T2	S2T1	9	12	26
S2T2N	S2T1	9	4	
S2T3	S2T1	10	13	28
S2T4	S2T1	10	8	23

[2] In these cultures the series number is given first. A 'twin' culture is indicated by the letter 'T' and a culture of single animals by the letter 'S.'

This series, in general, was much less vigorous than series I, although the structure of the twins was identical with that in the first series. It will be noted that the subculture of single animals (S2T2N) showed much less vitality than the twin subcultures. The chief importance attached to this series lies in the fact that it arose by the fusion of single animals which were descendants of a twin subculture (T15).

Series III. This series was started from a twin isolated December 16, 1917, from a depression slide which contained the progeny of a single 'normal' animal originally isolated for the purpose of studying the conditions under which cannibalism took place. From this slide three cannibals had previously been isolated, and at the time the twin was found, three attached pairs and sixteen apparently normal animals were present. The pedigree of this twin was followed for six generations:

1st generation. 2 twins (one considerably separated).
2d generation. 1 twin, 4 single.
3d generation. 1 twin, 10 single.
4th generation. 2 twins, 20 single.
5th generation. 1 twin, 44 single.
6th generation. The remaining twin had separated to give two single animals. This is the only instance where a twin series ended with complete separation of all 'twins.' The single animals were carried for some time on stock slides and multiplied normally.

Series IV. On December 6, 1917, a cannibal animal was isolated from Petri-dish culture of animals from culture A and placed in culture medium on a depression slide. Among the descendants of this animal, about fifty in number, on December 18th, several pairs were observed, fused in a manner similar to that previously described in section 2 (pp. 16, 17). One of these pairs was peculiarly attached in the peristomial region of each animal, with the bodies somewhat twisted around each other. This pair, along with several others, was isolated, and on the following day it was found that the animals which were so peculiarly attached had become more firmly fused and now formed a typical twin.

This twin divided to produce twins and single animals as follows:
1st generation. 2 twins (100 per cent).
2d generation. 3 twins, 2 single animals (60 per cent).
3d generation. 4 twins, 8 single animals (33.3 per cent).
4th generation. 7 twins, 18 single animals (28 per cent).
(Dec. 22) 5th generation. 12 twins, 40 single animals (23 per cent).

Eight of these twins were isolated on depression slides to study their later history in small mass culture, the remainder being isolated as controls and carried on slides, singly, with daily changes to fresh medium.

It was found in the cases where the progeny of the twins were allowed to multiply on the slide that division went on fairly rapidly. In four days the average number of animals on each slide was twenty-six, the proportion of twins to single animals being 20 per cent. At this time quite a few pairs were observed to be sticking together, and on nearly every slide were one or more cannibals. Two days later, cannibals were observed on every slide. On January 2nd only two twins were seen, and on the next day these had disappeared. The average number of single animals on a slide was now twenty-five. Thus in twelve days under these conditions no twins survived, while those which had been carried on in individual slides were still dividing to give twins. Further experiments on this series were not attempted. This is, however, the only case in which definite evidence as to the method of origin of twin forms has been obtained, and as the transformation of this pair of single animals into a twin was somewhat unexpected, it is regretted that more observations were not made during the process.

Series V. The ancestor of series V was formed in a six-day-old stock culture of single animals coming from a cannibal ancestor which had been allowed to multiply on a depression slide. Isolation was made January 9, 1917.

The history of the first four generations follows:

1st generation.	2 twins	
2d generation.	4 twins	
3d generation.	7 twins,	2 single
4th generation.	11 twins,	12 single

A twin culture (S5T) of four lines was begun on January 10th, using the four twins in the second generation as ancestors for the four lines. On January 12th a subculture of single animals (S5TS) was begun from the single individuals given off from twins in the third generation. On January 19th, from a stock slide

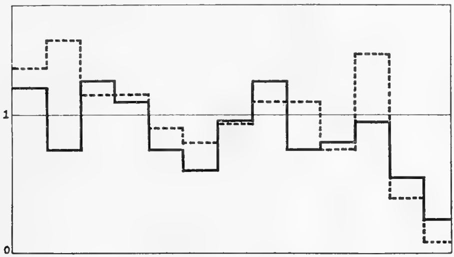

Fig. 9 Comparison of twin culture (S5T, continuous line) and subculture of single animals (S5S, broken line) derived from the twin culture, January 10, 1918, to March 16, 1918.

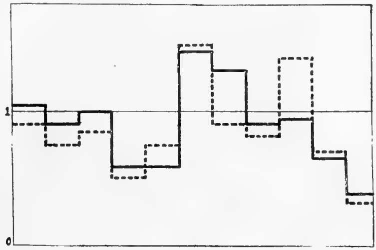

Fig. 10 Comparison of cannibal subcultures (S5C, continuous line) and non-cannibal subcultures (S5CN, broken line). January 20, 1918, to March 16, 1918.

from culture S5TS of four days' standing a typical cannibal and a non-cannibal animal were isolated, and the progeny of these in the second generation were carried in two subcultures, S5C and S5N, respectively. Curves showing the division rates for these four cultures are seen in figures 9 and 10. It will be noted that the division rates of the twins and the single animals

show a close agreement except at the beginning and near the close of the series. The curve for the cannibal line (S5C) shows a division rate very nearly the same as that for the non-cannibal line (S5CN), except that the division rate during the initial five-day period for the cannibal subculture is slightly higher than that of the non-cannibal—a result consistent with that obtained in section 4, part II, of this work. In general, the division rates are so similar that they might well represent four cultures carried for four entirely similar normal animals. The twin culture and all the subcultures derived from it died on March 15, 1918, the twin culture (S5C) being then in the sixty-second generation. No striking differences could be noted in these twins from beginning to end of the history of the culture either in appearance and behavior when living or in details of structure studied in stained preparations. In respect to cannibalism, it was found that it invariably took place in stock slides after a short period of multiplication.

Series VI. The twin beginning this series was isolated January 22, 1918, from a stock culture in a depression slide dating back to January 17th, made from stock of subculture 4B (p. 500, pt. 1). This twin gave the following:

2d generation. 2 twins
3d generation. 3 twins, 2 single

From the next generation the cultures (S6T and S6TS respectively) of four lines each were made from twin and single animals. Records of these cultures were carried in the case of the twins to the thirty-third generation and of the normals to the thirty-seventh. These lines at time of writing (March 26th) are still living and dividing as usual. In the curves for this series as well as those of subsequent series it will be noted that there is no striking difference in the division rates.

Series VII. The twin forming the starting-point of this series was isolated on January 25, 1918, from a ten-day-old depression- lide stock cu'ture of subculture 4B (p. 500, pt. 1). In the second generation it divided to give two twins and in the third three twins and two single animals, and from these, as in the previous series, cultures of twin (S7T) and single (S7TS) lines

were carried until the death of the animals in both cultures, which occurred on March 14, 1918.

The twin culture died in the thirty-first generation, the subculture of single animals in the thirty-seventh. Here the aver-

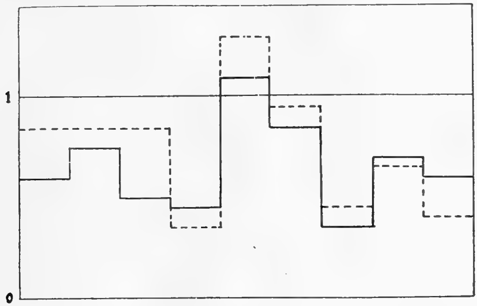

Fig. 11 Comparison of twin culture (S6T, continuous line) and of subculture (S6TS, broken line) of single animals derived from twin culture, January 25 to March 11, 1918.

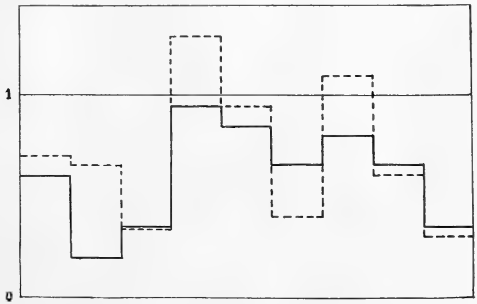

Fig. 12 Comparison of twin cultures (S7T, continuous line) and of subculture of single animals (S7TS, broken line) derived from the twin culture. January 28 to March 14, 1918.

age division rate of the single animals was slightly greater than that of the twins themselves. In general, however, the fluctuations in the division rate correspond to a remarkable degree.

Series VIII. A twin was isolated on February 9, 1918, from a Petri-dish culture of stock animals from subculture A4C, line 3, which was begun January 30, 1918. As usual there were present in the culture a considerable number of pairs as well as several cannibals.

Records of the division rate of this series were kept until March 11, 1918, when they were discontinued, the twins being then in the twenty-fourth generation and the single animals in the twenty-sixth. The cultures are at the date of writing still

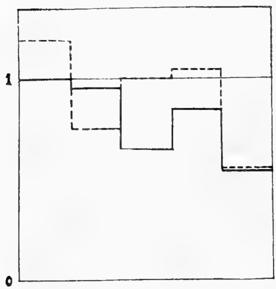

Figure 13

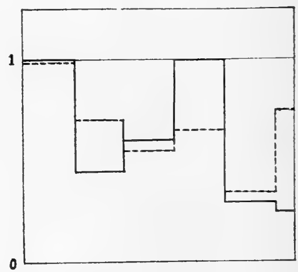

Figure 14

Fig. 13 Comparison of twin culture (S8T, continuous line) and of subculture of single animals (S8TS, broken line) derived from the twin culture. February 14 to March 11, 1918.

Fig. 14 Comparison of twin culture (S9T, continuous line) and of subculture of single animals (S9TS, broken line) derived from the twin culture, February 14 to March 13, 1918.

living and dividing. Essentially the same story is told by the curves for this series as in the others.

Series IX. The twin ancestor of series IX was obtained February 9, 1918, from a depression-slide culture from stock animals of line 3 culture A3N, begun February 2, 1918. The second generation gave one twin and two single animals, both of which died the following day. The twin divided to give two twins, both of which in the next generation gave one twin and two single.

Cultures of these were begun the day following, when the next fission occurred. Four lines in each were established, and these continued to breed twins and single animals respectively until the death of the series, which occurred March 3. Comment on the similarity of the division rates is unnecessary.

6. DISCUSSION

In part I of this study it was shown that, while no true process of syngamy was completed, there was reason to believe that the organisms periodically made abortive attempts to conjugate and that epidemics of cannibalism occurred. In the present paper it has been shown that the formation of double animals occurred under precisely the same cultural conditions as those in which pairing and cannibalism took place, i.e. this partial cytoplasmic fusion, which has proved, contrary to all previous observations on ciliates, to be permanent under suitable cultural conditions, arose while the animals were in a physiological state strikingly similar to that of true conjugants. Thus these three phenomena are in this organism apparently related, and it is believed that all three are but expressions of underlying physiological conditions which are usually expressed by syngamy.

The permanency of the union in the double animals is a notable feature, since in every series in which pedigrees were kept, with the single exception of series 3 (p. 143), twin animals were present until the series ended with the death of all the lines or until the cultures were discarded. Further, it is noteworthy that the 'miscible' condition of the protoplasm which made possible the formation of a twin animal persisted as long as the animal lived. That such was the case was demonstrated during the separation of the two daughter cells in fission. At this time there invariably occurred, in contrast to the final sudden separation of the two similar cells of the normal single animal, the pulling out of a relatively long thin cytoplasmic strand, enabling the anterior cell to tow the posterior cell along for a considerable period. In the case where a double animal separated by actual pulling apart of the two components, a similar drawing out of a cytoplasmic strand was observed, although in this case the con-

necting strand was always relatively shorter and thicker (fig. 21). Such a 'miscible' condition was present in the animals (part I) which simulated conjugants and in the cannibals, but the typical physical condition was in these cases always quickly resumed if the pairs were separated or the cannibals isolated and allowed to divide, the daughter cells in both cases being always constricted off sharply from each other with no pulling out of a cytoplasmic strand.

A more striking contrast was seen when twins divided by fission to give both twins and single animals, as the latter invariably divided in the manner usual in the typical single animal. It is therefore evident that the environment, although it was probably the principal factor in bringing about the pseudoplastogamic union, was not responsible for the continuance of the state of protoplasm necessary in such union. These results lead to the conclusion that, since the 'miscible' state of protoplasm continued only so long as the twin state persisted, it was the intimate union of the protoplasm of each component with the consequent possibility of protoplasmic interchange which was responsible for the permanency of the twin condition.

In other words, once a twin animal is formed the condition of protoplasm necessary for the maintenance of this union is transmitted to its progeny. This 'miscible' quality of the protoplasm of the double animal apparently acquired by the cells just previous to their union is clearly transmitted from generation to generation. Thus the inheritance of the 'miscible' condition in twin animals conforms with the conditions postulated by Jennings ('08, pp. 628, 629) as necessary for the inheritance of an acquired characteristic. The selection experiments showed unmistakably that the ability to remain united was possessed by some individuals in a greater degree than in others. A comparison of the pedigrees of subcultures T4 and T44 (fig. 8) as well as a study of figure 7 leaves little ground for doubt that there was a strong probability of obtaining a strain of double animals in which all the progeny would breed true for an indefinite number of generations, that is, no single animals would be thrown off by the double strain.

Further, the conditions necessary for the production of a race which at some time in its life-history will possess a dorsal spine (Jennings, '08) are, in a manner, realized in each series of twin animals. A twin frequently divided to give a typical twin from the posterior end and from the anterior end a twin widely separated anteriorly. This twin invariably pulled apart producing two single animals with pronounced dorsal spines (fig. 21). It is true that these spines were always soon resorbed, but the production of such individuals occurred regularly throughout the life-history of each twin series, so that what might be considered the establishment of a 'youthful' characteristic was transmitted from generation to generation, though not visible in the adult cell.

In part I of this study the conclusion was reached that, given favorable environmental conditions, the race would live indefinitely not only without conjugation, autogamy, or endomixis, but apparently without the ability to undergo these phenomena. The same conclusion seems equally applicable to the various races of double animals or twins. It is, of course, impossible to continue to breed the twins indefinitely in small mass cultures, since twins are invariably eliminated when bred in competition with single animals. It should be noted, however, that in the case of the longest-lived twin culture (AT) the twin forms outlived the culture of single animals (A) from stock of which they were derived.

Since the formation of double animals which reproduce consistently by fission to produce similar progeny for many generations under suitable cultural conditions is nowhere recorded in the annals of the Ciliates, it may be suggested, perhaps, that they are abnormal and, further, that the race on which the experimental study has been made is itself abnormal, inasmuch as all the individuals of the race are amicronucleate. If, however, the term abnormal is used to connote a pathological condition, its application to the phenomena described is highly misleading. The phenomena which have been described, i.e., pairing, cannibalism, and formation of double animals, are entirely 'normal' so far as this particular race is concerned, since such phenomena

have occurred with predictable regularity throughout the whole period of the study. The forms described are, indeed, atypical, judged by our present knowledge of the morphology and life-history of hypotrichous ciliates, but one may doubt whether this is broad enough to permit one to consider previously unknown phenomena as abnormal. Certainly, this race from every point of view is vigorous. The cannibals show at least as great reproductive ability, while the twins also, although apparently not able to compete with single individuals under the conditions of the experiment, are fully as vigorous.

Since the double animals are always eliminated in a short time when allowed to breed in a mass culture, the permanent establishment of such a form in nature is highly improbable. It is therefore considered that such twin formation is but one feature of the life-history of this amicronucleate race;—the result of an attempt to conjugate which is abortive probably because the organisms lack the nuclear constituents in a form for the carrying out of this process—it having been demonstrated (part 1), it is believed, that this race does not possess idiochromatin morphologically segregated as micronuclei.

7. SUMMARY

1. In cultures of an amicronucleate race of Oxytricha hymenostoma (part I), under conditions similar to those in which syngamy usually takes place in hypotrichous forms, there is a strong tendency for the formation of double animals or 'twins' by plastogamic dorsal fusion.

2. Twins, morphologically, have all the structures possessed by two single animals.

3. Twins reproduce giving, *a*) two pairs of twins exactly similar to the parent; *b*) from the anterior portion of the parent, a twin which pulls apart to produce two single animals, typical, except for the temporary presence of dorsal spines; from the posterior portion of the parent, a typical twin; *c*) from the anterior portion of the parent, two typical single animals; from the posterior portion, a typical twin. The components of a twin may pull

apart completely, producing two single animals, typical, except for the temporary presence of dorsal spines.

4. Twins may form, *a*) from normal strains in which no such forms have previously occurred, i.e., since isolation of original ancestor; *b*) from descendants of single animals arising from twins; *c*) from progeny of cannibal animals.

5. Favorable environmental conditions are necessary for the continued existence of twin forms; i.e., in stock and mass cultures in competition with single animals twins do not survive.

6. By selection a striking increase in the percentage of twins, produced in a pedigreed culture from a single twin animal, has been obtained.

7. The division rate of twin races is similar to that of normal single animals.

8. The miscible condition of the cytoplasm which leads to the formation of a twin animal continues as long as the animal lives; i.e., this condition is transmitted from parent twin animal to its progeny. In the case of single animals derived from twins and kept under identical environmental conditions, this miscible condition is quickly lost.

9. If twin animals are bred under favorable conditions (see 5 above) the union is usually permanent. A pedigreed strain (series I) of twins has been bred for one hundred and two generations, and it is believed that if suitable environmental conditions could be secured, the twin strains could be bred indefinitely.

10. As pairing, cannibalism (part I), and twin formation occur among animals in a similar physiological condition, it is believed that these three phenomena are but expressions of an abortive attempt to undergo the process of syngamy, which fails probably because the organism lacks the nuclear constituents in the proper form for the completion of this phenomenon—it having been demonstrated (part 1), it is believed, that this race does not possess idiochromatin morphologically segregated as micronuclei.

11. The inability of the organism to consummate syngamy clearly has no effect on the viability of the race.

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For additional literature, see part I of this study

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PLATE

PLATE 1

EXPLANATION OF FIGURES

The microphotographs were taken from permanent preparations stained with Delafield's hematoxylin and counterstained (unless otherwise specified) with tetra-brom-fluorescic acid. The same magnification (525 diameters) was used in all cases.

15 A twin in fission (somewhat flattened). The peristomial membranelles of three of the components are visible, those of the fourth are out of focus. Macronuclei show typical structure. (Del. hem. only.)

16 A typical twin. Side view.

17 to 21 These figures illustrate the process of separation of a twin to form a single animal. In figure 17 a twin is shown with slightly greater separation anteriorly than usual. Figure 18 shows a twin, similar to that shown in figure 17, in fission. The macronuclei here are somewhat fragmented. Figure 19 shows a later stage in the process of separation (ventral view). Both components are, during this stage, able to creep on the substratum. (Del. hem. and eosin.) Figure 20 illustrates the pulling out of a cytoplasmic strand from the posterior dorsal surfaces of each component. Figure 21 shows a single animal with the dorsal spine resulting fron the breaking of the cytoplasmic connection in a separating twin.

22 A twin showing clearly the fusion of the posterior macronuclei of each component. The anterior macronuclei are also fused, but are slightly out of focus.


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Resumen por el autor, H. Saxton Burr.
Universidad Yale, New Haven.

La transplantación de los hemisferios cerebrales de Amblystoma.

El autor ha transplantado los hemisferios cerebrales y las placodas nasales de Amblystoma en la región de los miembros de tal modo que en uno de los casos la placoda nasal cicatrizó en el ectodermo superficial mientras que en el otro se alojó en el mesenquima subectodérmico; el telencéfalo en ambos casos quedó profundamente alojado en el mesodermo. En estos experimentos el telencéfalo quedó sometido a la acción de dos condiciones de estimulación periférica. Bajo la primera condición fué estimulado por la presencia de la placoda nasal, capaz de actividad functional. En la segunda, las fibras centrípetas del nervio olfatorio, cuyas terminaciones periféricas no pueden funcionar, fueron las que susministraron el estímulo. Los resultados de estos experimentos indican que el factor que produce la fase de crecimiento en el desarrollo nervioso es el crecimiento interno de las neuronas, mas bien que la actividad funcional de un órgano terminal. Incidentalmente notó el autor que un vaso sanguíneo contiguo a la pared ependimaria del ventrículo produjo un plexo coroideo en la cavidad ventrícular del hemisferio transplantado.

Translation by José F. Nonidez
Carnegie Institution of Washington

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
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THE TRANSPLANTATION OF THE CEREBRAL HEMISPHERES OF AMBLYSTOMA[1]

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NINE FIGURES

An interesting phase of the problem of differentiation in the central nervous system is that of the rôle played by functional activity in the development of the neurone after the organ-forming period has passed. Harrison, Lewis, Bell, and others have shown that embryonic cells of the nervous system will multiply and differentiate in strange surroundings, apparently irrespective of their normal connections. In many instances the differentiation simulated more or less completely the normal morphology of the part from which the cells were taken. In other words, not only was there a differentiation of embryonic nerve cells into neurones, but also the neurones were grouped as they are in the adult brain. Whether such nervous tissue passed beyond the first period of differentiation into the second period of growth has not been indicated.

In the experimental study of the first phase in the history of a neurone there has been no evidence to show whether or not the neurone so differentiated was capable of functioning. If function in this particular instance means the transmission of an impulse along the neuraxis, it is practically impossible to determine positively whether neurones in strange surroundings and without their normal connections do transmit impulses and whether neurones so placed ever progress beyond the primary differentiation phase into the growth phase. This being true, it is necessary to determine what the factor which stimulates the neurone to further growth really is. Two possibilities suggest

[1] I am indebted to the Loomis Research Fund of the Yale University School of Medicine for the materials used in this series of experiments.

themselves. In the first place, it has been shown (Burr, '16) that the absence of a peripheral end-organ prevents the growth period in that portion of the central nervous system with which it is normally connected. This suggests that functional activity is the factor, or, in other words, that the passage of olfactory stimuli along the olfactory nerve serves to complete the development of the cerebral hemisphere. In addition to the above, it may be that it is necessary for the complete growth of the hemisphere that there be a pathway of discharge for the stimuli as well as a system by which stimuli may be received. In the second place, it is possible that functional activity does not enter into the problem at all, for it may be that the neurones of the cerebral hemisphere complete their development as a result of the ingrowth of the olfactory nerve regardless of the transmission of olfactory stimuli, the mere fact of the connecting up of the telodendria of the primary olfactory neurones with those of the secondary neurones serving as the factor necessary for the further growth of the hemisphere.

With the object of finding some data as to which of the above factors is operative, a number of experiments were performed on Amblystoma larvae in the spring of 1917, and repeated in the spring of 1919. Three types of experiments were carried out. In the first, the right cerebral hemisphere and the right nasal placode were transplanted to the region just posterior to the right limb so that the nasal placode was buried beneath the epidermis. In the second, the same structures were transplanted in the same way except that in this instance the olfactory epithelium was healed into the skin on the surface with the telencephalon deep in the tissue beneath it, maintaining in so far as possible the same relation between hemisphere and placode as exists in the normal position. In the third, a thin block of celloidin or of paraffin was healed into a cut made across the junction of the diencephalon and the telencephalon on the right side. These experiments created the following conditions:

In series I the nasal placode was healed in beneath the epidermis, thus preventing functional activity of the end-organ. Since in series II the nasal placode was healed in at the surface

and connected with the telencephalon, functional activity of the olfactory epithelium was possible, while discharge of peripheral stimuli from secondary to tertiary centers was prevented by the removal of the telencephalon from the rest of the brain stem. In series III the same conditions obtained as in series II, though reached by a different method.

In the course of the above experiments it developed that in addition to data on the problem just outlined, interesting pictures would appear of the fiber-tract organization of the cerebral hemisphere which had been removed from the possibility of tracts entering it from the lower centers of the brain stem. Also it became evident that some light would be thrown on the formation of the choroid plexus and on the nourishment of the transplanted structures in strange surroundings.

The experiments of series III were subsequently discarded, since it was found that the presence of a foreign body in the brain substance resulted in the rapid proliferation of the cells in the immediate vicinity of the block, forming many multiple cavities with a marked deformation of the walls of the neural tube. This made it impossible to analyze with any surety the effect of the operation on the hemisphere so isolated. The formation of 'Polymyelie' has been noted frequently in the literature, notably by Waelsch ('14), who produced multiple cavities experimentally in the spinal cord of chick embryos by means of knife thrusts piercing the wall of the central canal. This tendency of nervous tissue to proliferate aberrantly was noted frequently in the course of these experiments, hence great care was necessary not to injure the wall of the cerebral hemisphere in transplantation. That sections of the central nervous system can be transplanted without the formation of such pathological manifestations was shown by Hooker ('15). The experiments of series I and II have shown that the operation is entirely feasible, though very slight injury will produce all manner of distortions.

RESULTS OF EXPERIMENTS

In reporting the results of the experiments outlined above, there are a few general facts which it will be well to consider first. Figures 1 to 9 show three typical levels, each, from normal, series I and series II cerebral hemispheres in parallel columns. It is evident in general that transplantation by removing the hemisphere from its normal surroundings distorts to some extent the general outline of the transplant. The roughly semicircular outline is changed to an oval one, with considerable lengthening of the vertical axis and shortening of the transverse diameter. Concomitant with this is the narrowing of the ventricular cavity. The probable cause of these slight deformations is the fact that the hemisphere is removed from the enveloping cartilaginous covering, and placed in a portion of the body wall which is relatively thin, thereby bringing considerable lateral pressure to bear upon it.

The most striking fundamental difference between the transplanted and the normal hemisphere lies in the evident reduction in mass of the central gray. This diminution is evident at all levels of the telencephalon, although less marked, perhaps, at the posterior pole. As a result, there is an apparent increase in the number of peripheral fibers, although, as will be shown later, this increase is only apparent, as there is a noticeable decrease of fibers in certain regions.

In considering the problem of the factors involved in stimulating growth in the growth period, a consideration of figures 1 to 9 will show that little or no stimulating effect is produced by the functional activity of the nasal placode. For it is evident that a hemisphere without a functionally active end-organ, critical levels from which are shown in figures 4 to 6, is as completely organized as that in which the nasal placode is capable of function (figs. 7 to 9). That this is so may perhaps be better shown if one analyzes more completely the detailed organization of the hemispheres.

In order to understand clearly the effect of isolating the cerebral hemisphere by transplantation, it is necessary to refer to the

normal structure as worked out by Herrick in 1910. Briefly stated, the organization of the telencephalon is this. From the olfactory bulb, running caudal to the dorsal body of the anterior olfactory nucleus and to the posterior pole of the hemisphere is the tractus olfactorius dorsolateralis (fig. 1). A ventral division of this tract, the tractus olfactorius ventrolateralis, runs caudad intermingled with the lateral forebrain tract to the so-called corpus striatum opposite the anterior commissure. Some of the fibers of this tract in all probability enter the anterior olfactory nucleus. The lateral forebrain tract, above mentioned, consisting of ascending and descending fibers, runs between the olfactory bulb and the anterior olfactory nucleus and thalamus via the anterior commissure (fig. 2). On the median side of the hemisphere the tractus-olfactorius dorsomedialis runs from the olfactory bulb caudad to the dorsomedian part of the hemisphere, the area designated by Herrick as the primordium hippocampi (fig. 3). Ventromedially the tractus olfactorius ventromedialis, consisting of ascending and descending fibers, runs from the olfactory bulb to the hypothalamus. The pallial portion of the hemisphere, owing to its relative isolation from the rest of the brain stem by the diencephalic flexure, is connected with the brain stem by two relatively small groups of fibers. The first of these, the columna fornicis, runs from the anterior boundary of the primordium hippocampi first ventrad then caudad to the hypothalamus. The second of these is the stria-medullaris, running from the dorsomedian region of the primordium hippocampi through the commissura pallii anteriora to the pars ventralis thalami.

Two nuclei are apparent in the hemisphere, one laterally just posterior to the anterior olfactory bulb, the anterior olfactory nucleus (nucleus olfactorius anterior) and a second medially, the nucleus medianus septi, situated in the ventro-anterior part of septum ependymale (fig. 2).

As would be expected, the isolation by transplantation of the cerebral hemisphere precludes the development of nerve fibers which reach the hemisphere from lower parts of the brain stem. That is to say, the ascending fibers of the median and lateral

forebrain tracts are not present, but we do find the primary and secondary olfactory tracts more or less well differentiated.

We find, then, in the transplanted hemisphere the following tracts and nuclei to be present. As in the normal hemisphere, running caudad from the olfactory bulb is the tractus olfactorius lateralis, some of whose fibers enter the anterior olfactory nucleus and some of which proceed to the posterior pole (fig. 4). There is also a tractus olfactorius ventrolateralis running from the olfactory bulb caudad to the anterior olfactory nucleus, where apparently most of the fibers end (figs. 5 and 6). The lateral forebrain tract, a large tract in the normal hemisphere, is, in the transplanted hemisphere, reduced in size owing in part to the absence of any ascending fibers (figs. 3 and 6). The fibers of this lateral forebrain tract which are present in the transplanted hemisphere start from the lateral olfactory nucleus and run through the pars ventrolateralis hemisphaerii to the posterior pole of the hemisphere and there end. Running from the olfactory bulb ventromedially is the tractus olfactorius medialis. This tract in the transplanted hemisphere starts as a fairly large bundle of fibers running ventromedially into the region of the nucleus medianus septi, where it becomes very much reduced in size, finally to end. In an embryo killed thirty-seven days after the operation, this tract runs caudally to the primordium hippocampi. The two fiber tracts remaining undiscussed connecting the pallial region with the diencephalon are difficult to unravel in the transplanted hemisphere. There is apparently the beginning of a columna fornicis in a twenty-day embryo, since a number of fibers can be traced from the anterior boundary of the primordium hippocampi for a short distance ventrally close to the ependyma of the ventricle. Of the stria medullaris complex no trace was found.

From the above findings it is evident that Herrick's division of the hemisphere into four columns corresponding to the divisions of the lower parts of the brain stem is entirely correct. For we find that in transplanting the hemisphere we prevent the entrance of any ascending fibers and as a result find the ventral half of the hemisphere much less differentiated than normally (figs. 2

and 5, and 3 and 6). The reduction in size, which is the only apparent distortion, is due to the lack of the ascending fibers. But the dorsal half of the hemisphere, whose functioning is entirely olfactory, is practically completely developed with the exception of the tracts connecting it with the rest of the brain stem.

Of the three differentiated regions of the central gray, the anterior olfactory nucleus, the nucleus medianus septi, and the primordium hippocampi, the first is the only one normally differentiated. The nucleus medianus septi is reduced in size relatively, as is also the primordium hippocampi.

In a previous paper (Burr, '16) it was shown that in the absence of the olfactory nerve entering the hemisphere, the latter nevertheless showed all the typical differentiation of the central gray, although reduced in size absolutely. The type of operation there reported did not preclude the entrance of ascending fibers from lower centers in the brain stem. Hence it is reasonable to suppose that a part of the differentiation of the nucleus medianus septi and the primordium hippocampi is due to the ingrowth of centripetal fibers.

Herrick ('10, p. 424) suggests that the nucleus medianus septi arises through the wandering into that region of neuroblasts from other areas. From the above it seems more probable that this specialized area of the central gray comes about through proliferation of neuroblasts already present as a result of the stimulus given by the ascending fibers.

It is evident, then, that the factor which produces the second phase of nervous development in the telencephalon is not the functional activity of the nasal epithelium transmitted through the olfactory nerve, but rather the stimulus afforded by the actual ingrowth of neuraxes into the wall of the hemisphere. Only in the very young stages of development, however, do the olfactory neurones actually come into intimate relationship with the cell bodies of the secondary olfactory neurones. In the older stages the primary neurones interlace in the glomeruli with the secondary neurones at a considerable distance from the cell bodies from which their dendrites arise. This would seem

to indicate that the cell bodies migrate or are drawn caudally in the process of development. That this is not so I hope to show in a paper to be published later. Rather, in the process of differentiation, it is the glomeruli that move through the growth of the dendrites of the secondary neurones. When the nasal placode is removed the secondary olfactory neurones develop during the first phase of nervous differentiation. Failing, then, to make connections with the telodendria of their receptors, the growth phase does not ensue. When the placode is present, connection is established and further development follows.

In a previous paper (Burr, '16 a) the statement was made that "the presence of the latter (nasal placode) acts as a stimulus to the regeneration of a new telencephalon through the ingrowth of the olfactory nerve." The present experiments indicate the olfactory neurones can stimulate not only regeneration, but can also carry through the second or growth phase of nervous development.

As a secondary aspect of the operation it was noted that the transplants in every case showed a healthy development. The size of the transplant was in the main comparable to the normal. For instance, measurements made of a normal telencephalon showed an anteroposterior length of 740 μ; of a series I, 1080 μ, and of a series II, 800 μ. Evidently then, the hemisphere, though removed from its normal blood supply, was able nevertheless to obtain the materials necessary for growth. Under normal surroundings, the hemisphere is supplied with blood from two sources; first, the blood-vessels of the pia mater, and, second, the modification of the pia and ependymal wall, the choroid plexus. When the telencephalon is transplanted apparently the anlage of the pia is transferred also, and hence we find pia blood-vessels penetrating the brain substance. The derivation of these blood-vessels is any adjacent arteriole or series of arterioles. When an arteriole of sufficient size comes into contact with the thin membranous wall that forms the medial boundary of the transplanted hemisphere in the region that normally gives rise to the choroid plexus, we find such a plexus formed (figs. 6 and 9).

SUMMARY

The results of the series of operations may be summarized as follows:

1. Transplantation of the cerebral hemispheres indicates that the factor which stimulates the growth phase of nervous development is not the functional activity of the end organ, but rather the ingrowth of peripheral neurones.

2. In the transplanted hemisphere, the central gray is restricted particularly in the regions of the nucleus medianus septi and in the primordium hippocampi.

3. The absence of ascending fibers reduces the size of the lateral forebrain tract and practically prevents the formation of the columna fornicis and the fimbria complex.

4. A vascular pia mater is formed about the transplanted hemisphere and a choroid plexus may be formed from properly placed blood-vessels.

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PLATE 1

EXPLANATION OF FIGURES

All figures drawn with the aid of a drawing ocular at a magnification of 100 diameters from haematoxylin and erythrosin stained sections cut 10 μ thick. $\times$ 75.

1 Section through normal right cerebral hemisphere of Amblystoma host of a series I transplantation 180 μ caudad of anterior pole.

2 Section 190 μ caudad of figure 1.

3 Section 100 μ caudad of figure 2.

4 Section through transplanted telencephalon, series I, 140 μ caudad of anterior pole.

5 Section 160 μ caudad of figure 4.

6 Section 380 μ caudad of figure 5.

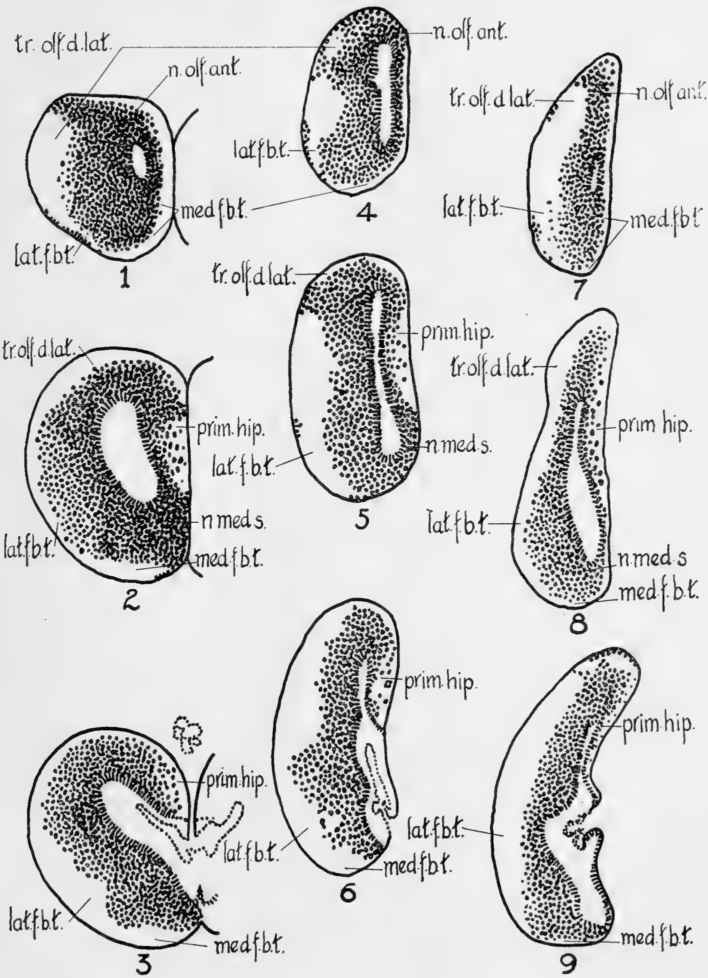
7 Section through transplanted telencephalon, series II, 140 μ caudad of anterior pole.

8 Section 180 μ caudad of figure 7.

9 Section 200 μ caudad of figure 8.

ABBREVIATIONS

lat.f.b.t., lateral forebrain tract
med.f.b.t., medial forebrain tract
n.med.s., nucleus medianus septi
n.olf.ant., nucleus olfactorius anterior
prim.hip., primordium hippocampi
tr.olf.d.lat., tractus olfactorius dorsolateralis


tr. olf.d.lat.
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med.f.b.t.
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1
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med.f.b.t.
4
tr. olf. d.lat.
n.olf.ant.
lat.f.b.t.
med.f.b.t.
7
tr.olf.d.lat.
prim.hip.
n.med.s.
med.f.b.t.
lat.f.b.t.
2
tr. olf.d.lat.
prim.hip.
n.med.s.
lat.f.b.t.
5
tr.olf.d.lat.
prim hip.
lat.f.b.t.
n.med.s
med.f.b.t.
8
prim.hip.
lat.f.b.t.
lat.f.b.t.
med.f.b.t.
3
prim.hip.
lat.f.b.t.
med.f.b.t.
6
prim.hip.
lat.f.b.t.
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La regulación asimétrica en embriones de anuros afectados de espina bífida.

En Bufo y Chlorophilus existen embriones en los cuales, cuando se inhibe la oclusión del blastoporo, aparece un proceso regulador de la asimetría. Cuando el embrión comienza a alargarse el blastoporo viene a situarse en una posición dorsal, como ocurre en el tipo familiar de espina bífida. Pero en vez de permanecer en esta posición y cerrarse por fusión gradual de los dos labios laterales a lo largo de la linea media dorsal, se desvía hacia uno de los lados del embrión hasta que uno de sus labios viene a situarse cerca o en la misma linea dorsal, mientras que el otro labio se mueve y queda colocado hacia la superficie ventral. El primer labio se organiza y, sin ayuda del otro, origina extensiones posteriores de los órganos axiales (notocordio, tubo neural, miotomos) que existen en el extremo cefálico del embrión. Un renacuajo de Chlorophilus que se estaba desarrollando de este modo fué criado hasta alcanzar el estado en el cual las branquias externas habían sido absorbidas, formándose las internas y la cavidad opercular.

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ASYMMETRICAL REGULATION IN ANURAN EMBRYOS WITH SPINA BIFIDA DEFECT

H. V. WILSON AND BLACKWELL MARKHAM

FIVE FIGURES

I

In fishes and amphibia it frequently happens that something interferes with the normal movement of the blastopore lip over the yolk. In these cases, familiar to embryologists, the anterior end of the axial body develops in front of the blastopore lip and is continuous behind with the two halves of the latter. Between these lateral halves in amphibia there lies bare yolk, whereas in fishes the exposed yolk mass may become completely or nearly covered in (Lereboullet's figures, '63) by a thin layer of tissue produced as an extension from the blastopore lips toward the median line. The embryos in the two groups are, however, essentially alike.

A survey of the facts leads to the following conclusions as to the ways in which such abnormalities may continue to develop:

1. Overgrowth may be merely slowed up, in which case nothing strikingly abnormal results.

2. The lateral halves of the blastopore lip organize into 'half-bodies.' Each half-body becomes a whole, and thus a double monster results. Hertwig ('92, p. 464) denies the possibility of this occurrence. But it was established as a fact by the observations recorded in Lereboullet's classic paper ('63; cf. especially Lereboullet's figs. 26 and 27, pl. III, and figs. 28 and 29, pl. III). Roux maintained it ('88, p. 516) and Endres further established it ('96, p. 541). Lereboullet actually observed (loc. cit. passim; cf. pl. 2, fig. 6) in a number of cases the gradual fusion of the two bodies of a double embryo (formed perhaps in other ways than the above) into one body. This, then, would be one way open

to such an embryo toward normality. Another way would be complete division.

3. The lateral halves of the blastopore lip organize into half-bodies, and these come together symmetrically in the midline to form a single embryo.

4. The lateral halves of the blastopore lip organize into half-bodies, but one atrophies while the other gives rise to an entire trunk. Or one lip fails to organize, while the other does so, first into a half-, then into a whole trunk. The two variants intergrade.

This paper describes two cases which fall in the fourth group.

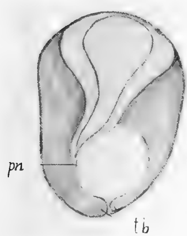
II

EMBRYO OF BUFO LENTIGINOSUS WITH ASYMMETRICAL REGULATION

Eggs of Bufo had been artificially inseminated February 27th, and from a culture the great bulk of which were developing normally, the embryo in question was picked out March 4th. In this embryo (fig. 1), which has begun to lengthen, the neural plate is conspicuous, but the yolk plug is still large. The medullary folds are both continued backward on the left side of the blastopore, ending posteriorly in a little projection, *p.n.*, which is distinctly lifted above the neighboring surface. The axis of the neural plate is thus not straight, but posteriorly inclines somewhat to the left, while the blastopore has been pushed over toward the right. In the middle of the posterior (ventral) lip of the blastopore, and therefore at some distance from the posterior end of the neural plate, there are two very small knobs, *t.b.*, representing the tail buds.

The embryo was sectioned, and figure 2 represents a section taken transversely through the posterior end of the neural plate and therefore across the yolk plug. The archenteron, *arch*, is large. The notochord has formed. The neural groove is present, with a medullary fold, *lmf* and *rmf*, on each side of it, the left one the larger. The gastral or notochordal mesoderm, *nc.m.*, is distinguishable on each side of the notochord, on the right pass-

ing at once into the tissue of the blastopore lip, on the left thinning out at a little distance from the notochord into a sheet, which farther outwards is not distinguishable from the dorsolateral wall of the archenteron. Peristomal mesoderm, *p.m.*, has begun to differentiate on the other side of the embryo extending ventrally from the right blastopore lip, *r.b.l.* Otherwise this lip, the right one, shows no differentiation.



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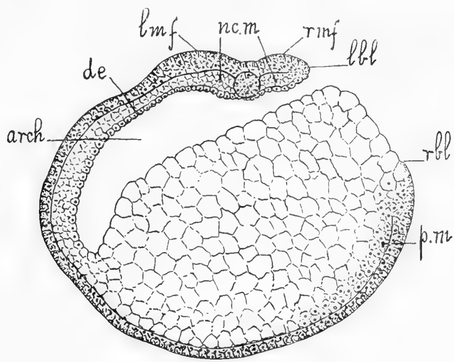

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Fig. 1 Bufo embryo. Dorsal view. *pn*, posterior end of neural plate; *tb*, tail buds. × 18.

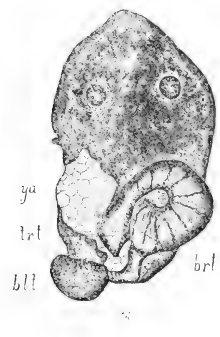
Fig. 2 Transverse section of embryo shown in figure 1, through the blastopore. *arch*, archenteron; *de*, dorsal entoderm; *lbl*, left blastopore lip; *lmf*, left medullary fold; *nc.m*, gastral mesoderm; *p.m*, peristomal mesoderm; *rbl*, right blastopore lip; *rmf*, right medullary fold. × 40.

It is clear that in this embryo a full set, not a half set, of axial structures had begun to develop on the left side of the blastopore. In this differentiation of the axial body, the right lip had no share.

CHOROPHILUS LARVA WITH ASYMMETRICAL REGULATION

The frog larva next to be described (figs. 3, 4, and 5) represents fairly well a later stage of the embryo shown in figure 1, supposing that the asymmetrical regulation begun in the latter had gone on. This embryo appeared in a batch of normally develop-

ing eggs of Chorophilus feriarum which had been artificially inseminated February 15th. It was early singled out by Mr. T. E. Rondthaler as one in which the blastopore did not close, and was kept under more or less continuous observation. It elongated and became somewhat flattened dorsoventrally, the yolk plug occupying a median dorsal position in the posterior part of the body. Immediately behind the yolk plug two tail buds, one left, one right, appeared. They both early acquired a spiral



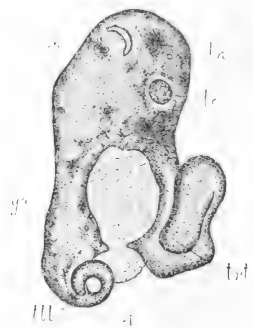


Fig. 3 Chorophilus larva. Dorsal view. *blt*, base of left tail; *brt*, base of right tail; *trt*, tip of right tail; *ya*, exposed yolk area. × 25.

Fig. 4 Ventrolateral view, from the left, of larva shown in figure 3. *le*, left eye; *ln*, left nostril; *m*, mouth; *tlt*, tip of left tail; *trt*, tip of right tail; *ya*, exposed yolk area. × 25.

curvature in different planes. External gills, which are small in this species, developed. With their disappearance and the steady increase in size of the head and trunk region, the yolk plug gradually shifted over toward the left side of the body. One tail, the left, was thus carried into a position which was distinctly ventral (fig. 4). The other tail, the right, which gradually became much the stouter, took up a position which was distinctly dorsal, its basal part directly in line with the median longitudinal axis of the trunk (fig. 3).

By this time the larva showed well-developed eyes, mouth, and nostrils, and was about 2 mm. long with a greatest breadth of 1 mm. It did not die naturally, but was preserved, March 8th. At that time it seemed to be in good health with a fair prospect of living on. Sections showed that internal gills and the opercular cavity were well developed. The right tail had relatively large dorsal and ventral fins in its posterior part, and at its tip the left tail likewise had fins. The exposed yolk area

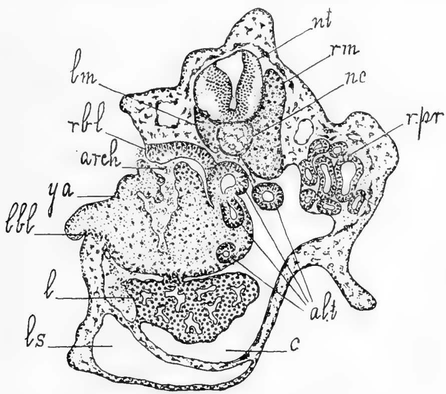


Fig. 5 Transverse section of larva shown in figures 3 and 4, through anterior end of yolk mass. *arch*, archenteron; *alt.*, tubes representing an alimentary canal in process of formation; *c*, coelom; *l*, liver; *lbl*, left blastopore lip; *lm*, left myotome; *ls*, lymph sinus; *nc*, notochord; *nt*, neural tube; *rbl*, right blastopore lip; *rm*, right myotome; *rpr.*, right pronephros; *ya*, exposed yolk area. × 67.

measured in length about one-half the total length of the body. Internally the yolk mass was found to occupy slightly more than this ratio.

A study of the internal anatomy as made out in a series of transverse sections gave the following facts: The brain, eyes, auditory sacs, nostrils, mouth, pharynx, gills (internal), opercular cavity, and heart showed nothing obviously abnormal. But in the trunk and tail region there were numerous results that had followed from the failure of the blastopore to close. We

take these up in order, as affecting the alimentary canal, spinal cord, notochord, myotomes, and pronephros.

Alimentary canal. The anterior end and the pharyngeal region of the alimentary canal have the usual anatomy, appearing as parts of a wide, straight passage. This comes to an end at the anterior limit of the yolk mass. The latter in this region is found to be excavated by numerous tubular channels, round which the cells are arranged in epithelial fashion. A few other similar tubes with less yolk in the cells lie at the surface of the mass (fig. 5). It is possible to trace some interconnections between these, and the tubes are perhaps all interconnected, representing an alimentary canal in the making. A remnant of the original archenteron persisting as a shallow slit-like cavity, which extends well into the yolk and connects with the exterior round the right lip of the blastopore, is present in this region (fig. 5, *arch*). It connects with, at any rate some of, the tubular channels above referred to. Possibly it forms the terminal, anal, part of the alimentary canal which is in process of differentiation. The posterior and much the greater part of the yolk mass remains as solid and compact and undifferentiated as in a gastrula stage.

It is clear that in this larva an alimentary canal was in process of formation by a method different from the normal. The departure is probably adaptive to the continued presence of the yolk mass. In the figurative language of vitalism, the embryo makes an effort to form an alimentary canal, although the customary road to that end is not open. Moving slightly away from vitalism, we seem to see the destined end of the ontogeny working backward as a cause, a philosophic idea which certain embryological writers (cf. Jenkinson's admirable book, '09, p. 20) have in recent years shown a willingness to adopt into our family of concepts concerning the processes at work in ontogeny. To invoke such retroactive influences may, as Jenkinson says, in the end prove necessary, but on the other hand it may not. The fact is that while the idea of individual adaptation or regulation in ontogenetic processes is now familiar to us, the detailed facts (comparative and experimental) of any particular set of cases

have only been touched upon in a preliminary, reconnoitering sort of way. Brilliant as the results are, they need to be interwoven with rich collections of special knowledge, which shall combine the data of experimental organogeny with tabulations of the various organogenetic methods practiced by sets of individuals, and races, of a species and by related species. Twenty-five years ago, one of the writers mapped out in a descriptive paper (Wilson, '94) this plan as affecting the study of the comparative embryology of sponges. The idea was to learn something, through comparison, of the kinds of changes that may be made in the ontogenetic processes of a race and the laws governing their appearance. The comparative study remains as important to-day as it was then, and the field is almost as virgin. In a word, before accepting this conclusion that the end sets in activity the means, we need to know in any particular case much more both about it and allied cases. Ontogeny we picture as an intricate series of events, each event setting free stimuli which bring about other events. If, then, a certain event does not occur, the embryo in respect to this point may, for all we know, fall back to a lower level, to a more generalized ancestral condition, in which it makes responses such as it would have made in former times. Or it is possible that steps in ontogeny not directly dependent on the occurrence of the inhibited event may continue to be made, one after another, and thus we may come out with an embryo in which only a part is missing, in which case the missing part might conceivably be supplied as if it had developed, had been lost, and now were being regenerated. All this, of course, is conjecture, but it is at least physiological.

Liver. The liver appears as a large cellular mass excavated by abundant sinuses which divide it up into cords. It is intimately connected, throughout most of its extent, with the yolk mass, shading off into the latter in places.

Spinal cord. The spinal cord in the trunk region, as far back as the base of the right tail, is symmetrical, one side having the same amount and arrangement of incipient gray and white matter as the other. At the base of the right tail, the spinal cord is continued into a tube with a much wider lumen. The wall of

this caudal continuation of the cord is markedly thicker on its morphological right side than on the opposite side. On the former side it consists of a high lining epithelium, outside which lie some rounded cells and then a little white matter, whereas on its left side the wall consists only of a layer of low epithelium. The tube narrows to one which still presents a relatively large lumen, but the wall of which is nearly uniform in thickness, consisting of a simple columnar epithelium. It extends nearly to the tip of the tail.

Summing up for the neural tube, it may be said that the neural tube of head and trunk is continued directly into the right tail. Elsewhere symmetrical, in the right tail it gives an indication of being a half-cord. In the left tail and along the left lip of the blastopore there is no sign of a neural tube.

Notochord. The cylindrical notochord of the posterior head region and trunk is continued directly into the right tail with no diminution in size. It extends the whole length of the tail, remaining cylindrical, but decreasing gradually in diameter in the usual way. In the left tail and along the left blastophore lip there is no sign of a notochord.

Myotomes. The myotomes of the trunk are as in figure 5. There are two series, but those on the right side, *rm*, are strongly developed, those on the left side, *lm*, very small. The series of strongly developed myotomes on the right side of the body is continued into the right side of the right tail, and the series of small myotomes on the left side of the body into a similar series on the left side of this tail. This disproportion in size, between the two series of myotomes in the tail, is as great or greater than in figure 5. The series of right-hand myotomes is continued almost to the tip of the tail. The series on the opposite side comes to an end shortly behind the basal part of the tail. In respect to the myotomes, then, the basal part of this tail is similar to the trunk (fig. 5), but the greater part is more distinctly a half-tail in that it lacks myotomes on one side. Doubtless these were developing from before backward, when the larva was killed.

The other, originally left, tail has a distinctly developed series of small myotomes on one side, the side turned away from the yolk mass (originally the left side). This extends from about the tip of the tail to its base and forward as a very small stripe for some distance, close to the morphological left lip of the blastopore, fading away anteriorly. It is evident, then, that the left lip of the blastopore had made steps toward organization. But the regulatory processes going on along the other lip made these steps futile.

Pronephros. The right pronephros, figure 5, *r.pr.*, is well developed and the right Wolffian duct extends backward through the trunk. What appears to be the left Wolffian duct is present near the left blastopore edge, about the middle region of the body, extending through a number of sections. The anterior end of this tube could not be traced. Thus whatever steps have been taken to form a left pronephros have not gone beyond the merest start.

In the embryo just described the axial organs (neural tube, notochord, myotomes) have not, it is clear, been built up by the fusion in the middorsal line of two halves. Instead, an asymmetrical method has been followed, the fundamental feature in which is the utilization of only one blastopore lip. The steps in this process are briefly as follows: 1) The exposed yolk area is shifted over toward the left side. 2) The right blastopore lip and the right tail bud are thus brought in line with the median axis of the head end. 3) The right lip organizes a backward continuation of neural tube, notochord, and the right series of myotomes, all continued into the right tail bud, which grows much larger than the left one. 4) After having established axial organs in the trunk region, the right blastopore lip grows for some little distance over the exposed yolk, thus coming to lie to the left of the median body plane. A series of myotomes is then organized along the left side of the notochord. 5) The left lip of the blastopore and the left tail bud make no more than the first steps toward organization.

The behavior and the anatomy of the larva at the time of preservation indicated that the process of establishing the normal

structure would have gone farther had the larva been allowed to live. The yolk mass was apparently being covered in through further extension over it of the blastopore lips. The mass itself had made some headway toward transformation into a part of an alimentary canal. The left series of myotomes would probably have extended itself to the tip of the tail (right tail), and would have continued to grow in size. With the continued dorsal extension of the coelom on the left side of the body (fig. 5), the conditions for the formation of a left pronephros, to match that already developed on the right side, would have been more nearly realized. Absorption of the left, small tail would have completed the steps by which, in spite of its failure to go through certain early phases of the normal cycle of changes, the embryo might in the end have acquired the type structure. We hope at some later date to have actual observations to report on the final stages in the restoration of such asymmetrically developing larvae, if indeed they do succeed in going through the entire process. It is to be expected that here, as elsewhere, in respect to the actual details, individuals will vary.

Cases essentially similar to the above have been recorded by Lereboullet ('63) for the teleosts. In the embryo shown in his figures 30 and 31, pl. III, one of the half-bodies degenerates, while the other develops into a whole body. This latter comes to lie in direct line with the head end. We thus get what is not far from a normal embryo, a lobe-like projection from one side and a split tail alone remaining to indicate the original duplicity. This conclusion, which Lereboullet draws, is made practically certain by the several embryos which he describes and which individually illustrate different stages in the process. Indeed, in the case of another embryo like his figure 31, Lereboullet was able to observe from day to day the gradual degeneration of one of the half-bodies. In another closely similar case, only briefly described, no. 55, loc. cit., p. 224, it would seem that only one blastopore lip became organized as a half-body, but Lereboullet is not very explicit here.

The frog embryos studied by O. Hertwig ('92) do not, as he points out, all conform to the symmetrical type of development.

For in some of them the organs develop in very unequal degree in the two lateral blastopore lips. This asymmetry is extreme in the embryo pictured by Hertwig in figures 15 and 16 of pl. XVI and figure 27 of pl. XVIII. In this individual development had gone so far that eyes and suckers had been formed. Nevertheless, nearly the whole of the dorsal surface was occupied by an exposed yolk area. The special point of interest is that one of the lateral blastopore lips was found to be not organized at all. The opposite lip, on the contrary, contained a small neural tube and notochord, which anteriorly were continued into the corresponding structures of the very short head end and posteriorly into a well-developed tail (half-tail or tail bud). Hertwig regards this interesting embryo merely as one in which the axial half-structures developed on one side and not on the other. He does not view or discuss it as illustrating a step in the direction of forming a whole trunk out of one blastospore lip. And indeed it may not illustrate such a step, although it obviously presents a close analogy to the Chorophilus embryo described above.

Endres and Walter ('95) described a frog embryo in which possibly a process was going on similar to that in our asymmetrical embryos. They, loc. cit., p. 41 and passim, find with Roux that half-embryos, developing from one of the first two blastomeres, restore more or less completely through 'postgeneration' the missing organs of the other side. In the case referred to (Hemi-embryo sinister: F, loc. cit., pp. 43 to 48) there is a large, dorsal, exposed yolk area. One blastopore lip is well organized and passes backward from the head end into a well-developed tail. The other, the 'post-generated' lip, is very imperfectly organized.

III

The common or typical regulatory method of establishing the type structure employed by embryos in which the closure of the blastopore has been extensively interfered with, is generally thought to be that in which both lips organize and come together symmetrically in the midline (group 3, p. 172). And there seems to be no doubt that this method is employed sometimes, although observations on its occurrence in an actual embryo are scanty.

Lereboullet records some direct evidence for the teleosts. In case no. 56 (loc. cit., p. 225, figs. 32, 33, and 34) he watched the actual development of the embryo from day to day. Anteriorly and posteriorly some fusion of the two half-bodies apparently took place, although as late as the seventh day the half-bodies were still separate throughout an extensive part of the trunk, and no further fusion took place during the remaining five days of the embryo's life. It is, however, probable that the now common view, which Lereboullet advanced, applies to this case and that under favorable circumstances the two half-bodies would have completely fused. The truth of this idea is made almost certain by cases like Lereboullet's no. 57 (loc. cit., p. 226), in which the half-bodies have been brought so close together as to be practically in contact, and his no. 59 (loc. cit., fig. 35), in which at the posterior end of the single trunk there is found a small dorsally situated aperture, interpreted as the remnant of the blastopore.

O. Hertwig, in his well-known paper ('92) on frog embryos exhibiting the spina-bifida defect, describes many such embryos in anatomical detail. He arranges them in a series which he interprets as representing the actual ontogeny. His series begins with the so-called 'ring embryos,' in which the exposed yolk area is very large and the part of the axial body lying in front of the blastopore lip very small—so small, indeed, that it includes no more or scarcely more than the anterior cerebral commissure. In such embryos he concludes with Roux ('88) that the lateral lips of the blastopore become organized into half-bodies, which gradually meet in the midline. And the individual frog embryos which he describes and arranges in a series represent, he thinks, successive stages in the actual ontogeny of such a 'ring embryo.' It is far from certain that all of the abnormalities, so arranged, really belong in a single ontogenetic series. Nevertheless, the data, recorded with admirable precision, doubtless justify the conclusion that sometimes the lateral lips do organize and come together in frogs, as Lereboullet had already claimed was the case in fishes. But Hertwig has no observations on the actual occurrence of this process in one and the same embryo.

In fact, direct observations on the occurrence of the symmetrical fusion of half-bodies are, as we have said, few and scanty. They lack detail. Nevertheless, Roux ('88, p. 443) has actually watched the gradual approximation of the lateral lips of the blastopore in frog embryos exhibiting the spina-bifida defect (designated by him, consistently with his theory, asyntaxia medullaris), until the blastopore had quite closed.

This symmetrical mode of development of spina-bifida embryos is looked on by many embryologists (cf. especially Roux, '88, pp. 443–444, and O. Hertwig, loc. cit.) as not fundamentally different from the normal embryology. The difference, according to these writers, is that, whereas in normal development the lateral blastopore lips come together before they are organized, in the spina-bifida embryos their fusion is so delayed that they become organized first. Thus the organization of the blastopore lips in a spina-bifida embryo is not looked on as an abnormal regulation that starts up when something checks the activity of the morphogenetic process by which the body is usually lengthened. It is, on the contrary, a process that is perfectly normal in itself, only out of place in time. This is the point of view of the concrescence theory of vertebrate ontogeny.

Undoubtedly, symmetrical spina-bifida embryos strongly suggest the idea of concrescence—so strongly, indeed, that they are thought of by some as almost demonstrating the truth of its occurrence in normal development. But this reasoning is obviously vicious, since it begs the question as to whether organization of the lateral lip is a normal process (although out of place in time) or a radically abnormal one. Such radically abnormal regulatory processes of course occur. Driesch and many others have made us far more familiar with them than were the older embryologists. And in establishing or restoring the type structure, their disregard of the customary mode of attaining that end is well known. Following this line of thought, we may entertain the idea that the organization of the blastopore lip in the embryos in question is a thoroughly abnormal process, as far away, although in a different direction, from the normal mode of forming the axial body as is the process, for instance, in those rare teleost

monsters, in which the blastoderm does not grow round the yolk, but remains small, while an embryonic body is developed extending completely through it from posterior to anterior edge (Kopsch, '04, p. 95, Taf. X, Fig. 118).

If the organization of the lateral blastopore lip is an unusual, abnormal process, spina-bifida embryos become no less interesting than they have been hitherto, since they tell us plainly that here is tissue which may be activated to develop, in very atypical fashion, into certain of the specific structures. From this point of view, such embryos may be thought of as arising in the following fashion. The axial body which normally forms in front of the blastopore lip is short. It grows in length especially at the posterior end which is carried backward, the growth being in part due to the incorporation of tissue belonging to the blastopore lip. The indifferent mass of tissue at the posterior end of this embryonic body is constantly being organized and added to the organs in front of it, some to neural tube, some to notochord, etc. Frankly abandoning deterministic theories which would see in the tail bud of a vertebrate embryo not indifferent tissue, but neural, notochordal, and other kinds of cells, we ask what brings it about that certain cells go to one, other cells to another organ? Plainly O. Hertwig and the thinkers of that school are right; it is position that determines the fate of the cells. Those behind the notochord become notochord. Those behind the neural tube become neural tube. What underlies this phenomenon? The answer seems clear: the already differentiated organ, notochord, e.g., exerts a controlling influence on the contiguous, indifferent cells behind it and *makes them into its like.*[1]

Now when the backward growth of the blastopore lip (to be construed as part of the general closure of the blastopore), and hence of the axial embryonic body, is prevented, what is the sit-

[1] Roux has discussed this kind of influence ("eine eigenthümliche ordnende und gestaltende Wirkung," 1888, p. 505 and passim) in the case of half-embryos of the frog produced by killing, or nearly killing, one of the first two blastomeres. In the formation, 'postgeneration,' of germ layers in the operated half, the organizing influence extends outward from the edge of each, already formed, germ layer of the normal half. This progressive differentiation of relatively indifferent stationary material, where the "differentiative stimulus passes from the al-

uation? The embryo finds itself unable to elongate directly, and yet continuous at its posterior end with two stripes of indifferent tissue. These are accordingly, under the general influence of the regulatory tendency, organized through the action of the structures in front, precisely as the growing tail bud is organized in the normal embryo. The difference is that in the spina-bifida embryo the entire stripe is there from the start, while the cord of cells, to which the backwardly growing tail bud gives rise, is formed and metamorphosed into its several derivatives gradually.

There are still other ways in which we may conceive of spina-bifida embryos as being formed. Kopsch ('96, '99, '04) and H. V. Wilson ('00), for instance, have held that it is not necessary to regard them as due to organization in situ of the blastopore lips, but that they may be produced as the result of a progressive splitting of the axial body. This explanation may well apply to some cases.

It is plain that spina-bifida embryos of themselves will not tell us whether they have been built up by normal or abnormal processes. If the blastopore lip organizes, we may claim with one school that it is a normal process, with the other that the lip has been activated perhaps in the manner described above. If it does not organize, as may happen, we may adduce this as proof that there is no normal impulse in it toward organization, or with the other school that the normal impulse has been inhibited. In the midst of this debating one does not forget, however, that the proximate, so-called practical, thing to do is to learn how to control the differentiation of the lip, to call out in it, or prevent perhaps, the formation of a neural tube, notochord, and somites.

ready differentiated cells" into the indifferent mass, falls in Roux's category of 'dependent differentiation' ('abhängige Differenzirung'). The directive force Roux designates 'an assimilative and differentiative action,' (loc. cit., p. 509). To it is applicable the term 'morphological assimilation' (Roux, '12, p. 28), which might be used, pending the coinage of an appropriate Greek word. Other embryologists had already postulated the existence of such an influence in normal development, but Roux, I believe, for the first time gave precision to the idea.

Whether in ordinary development there is a virtual concrescence of lateral blastopore lips in the midline, and eventual organization of these, is a question which is most definitely answered not by a study of monsters, but by analytical studies on the normal embryo. Some years ago one of the present writers (Wilson, '00, '01) published data which together with the work of O. Schultze (ref. in Wilson, loc. cit.), Assheton (ref. in Wilson, loc. cit.), Kopsch ('00 and earlier papers), Eycleshymer ('02 and earlier papers), Ikeda ('02), seem to make the concrescence theory untenable for the amphibian egg, since they necessitate the conclusion that a considerable part of the dorsal axial body is formed in situ, viz., in front of the dorsal blastopore lip where it first forms. That the posterior part of the dorsolateral wall of the embryo (gastrula) is produced by the backward growth of the corresponding part of the blastopore lip is admitted by everyone, but it is plainly arbitrary to construe this as a modified form of concrescence. All parts of the blastopore lip grow backward, dorsal, lateral, and ventral. The difference in the distance covered by dorsal and ventral lips in the frog is, to be sure, considerable, but this inequality is readily understood as a part of the general asymmetry in gastrulation—an asymmetry due to the acquisition and distribution of yolk in the egg, as Balfour long ago pointed out.

Likewise the data adduced for fishes by Morgan ('95), Kopsch (Kopsch's splendid study published 1904 contains references to his earlier papers), and Sumner ('00, '03) make it practically impossible to believe that concrescence normally occurs in these forms.

That the concrescence theory may contain truth as a phylogenetic theory, in other words, that concrescence may have actually occurred in the evolution of the distinctly bilateral metazoa from coelenterate-like forms, remains of course possible. And with richer and more precise knowledge, such questions doubtless will be taken up again in the future.

Lereboullet is often cited as an adherent—in fact, as the first promulgator—of the concrescence theory. But this is to read into his paper ('63) an interpretation that is not, I believe, war-

ranted. He recognizes that the fish body is first marked out as a projection reaching forward from the blastodermic rim. This 'tubercle,' in normal development as in double-embryo formation where two tubercles are formed, elongates to form a linear body ('bandelette embryonnaire'). Lereboullet speaks of the production of the original tubercle as due to a sort of 'vegetation' from the blastodermic rim (a description that is clearly not warranted). As to the processes involved in its elongation, he is not explicit. But it would seem that he means that the tubercle elongates by its own growth, possibly incorporating the neighboring tissue of the blastodermic rim (which no one would deny). By contrast, in his fourth kind of anomaly, what we call to-day the spina-bifida embryo, the original tubercle does not grow and elongate, but the lateral blastodermic edges organize and participate directly, as such, in forming the body. In fact, in his search for the growth processes that lead to his various anomalies, he gives the impression of having distinctly in mind the power of the embryo to proceed to the type structure in more ways than one. Whereas, some of the later embryologists, like O. Hertwig, assume for the moment at least that ontogeny always exhibits the same series of events, anomalies being due to retardation or acceleration in the occurrence of particular events. While undoubtedly many anomalies are produced in this way, there are as certainly others the causation of which is much more subtle and complex.

SUMMARY

Bufo and Chorophilus embryos occur in which, when blastopore closure is inhibited, an asymmetrical regulatory process comes into activity. Instead of the two lateral blastopore lips fusing in the midline, the blastopore is shifted over toward one side, and from a single lip a backward extension of the axial organs is produced. Such a tadpole was reared to a stage in which external gills had been absorbed, internal gills and opercular cavity formed.

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Resumen por el autor, Bennet M. Allen.
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Los resultados de la extirpación temprana de las glándulas timo de la larva de Rana pipiens.

El autor ha extirpado cortando, los esbozos de las glándulas timo de renacuajos de Rana de 8 a 8.5 mm de longitud, después de la cual los animales operados sanaron rápidamente a pesar de la grave operación doble que fué necesario practicar. Se han notado los siguientes hechos: 1) Las glándulas timo, desde su primera aparición, no ejercen influencia ni sobre el desarrollo ni sobre el progreso de la metamorfosis. 2) No son indispensables para la vida del animal en ningún estado del desarrollo, ni su extración parece causar una deficiencia marcada en el metabolismo general del cuerpo. 3) La extirpación de las glándulas timo no modifica en modo alguno ni la cualidad ni la marcha y grado de desarrollo de las gonadas. 4) Un estudio bastante incompleto de las glándulas tiroides de ranas desprovistas de timo no ha podido demostrar su anormalidad en ningún sentido. Se puede pues afirmar que las glándulas timo no ejercen influencia sobre las glándulas tiroides. 5) No se ha podido comprobar la existencia de modificaciones de los rasgos externos e internos como resultado de la ausencia de las glándulas timo.

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THE RESULTS OF EARLIEST REMOVAL OF THE THYMUS GLANDS IN RANA PIPIENS TADPOLES

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ONE FIGURE

It is a matter of common knowledge that we are much in the dark regarding the function of the thymus gland. Results of extirpation and of thymus feeding have been very conflicting. Gudernatsch ('12, '14) claimed that administration of thymus glands as food to Rana tadpoles retards metamorphosis and at the same time stimulates growth. It has seemed to the writer that Gudernatsch killed his tadpoles prematurely—that he should have carried out his experiment at least a month longer than he did. Romeis ('14) gained rather indifferent results upon this question. Swingle ('17), working in this laboratory, repeated these experiments with uniformly negative results. He used fresh thymus glands of young calves and found that his tadpoles thus fed all underwent metamorphosis at the usual rate and in typical fashion. Uhlenhuth ('18), in earlier researches, found that feeding mammalian thymus to larvae of salamanders caused actual increase in growth in some while others showed slight retardation. He concluded that this was due not to qualitative, but to quantitative factors in the food. Later experiments where the diet was exclusively made up of thymus-gland material showed a decided resultant retardation in growth and differentiation. He concluded that the thymus gland is deficient in certain substances essential to growth.

In a series of interesting experiments upon salamander larvae he produces tetanus by means of thymus feeding. He concludes

that this is due to the presence in the thymus gland of a tetany-producing substance. He fails to produce tetany by feeding thymus glands to frog tadpoles, and concludes that here the injurious influences of the thymus gland are counteracted by the parathyroid glands that here develop much earlier than in the salamanders.

The feeding experiments of Paton and Goodall ('14) upon young guinea-pigs and rats are also negative. These authors give an extensive discussion of the literature of the subject. There is a good discussion of this field in general in a recent paper by E. R. Hoskins ('18).

Many experiments have been made upon the extirpation of the thymus gland. A recent paper by Pari ('05) shows that this produces no result in the guinea-pig. One of the most widely quoted papers is that of Abelous and Billard ('96). These authors claim that extirpation of the thymus gland in the young or nearly mature frogs produced effects classified as follows:

1. Dynamic: Little resistance to fatigue—muscular feebleness.
2. Trophic changes: Skin discoloration, ulcerations.
3. Blood changes: Oedema, increase of serum.

Camia ('00) tended to substantiate these results; but Pari ('05) showed that these symptoms occurred in only a certain portion of his experiment. The majority of his frogs showed no symptoms of any kind as a result of thymus extirpation. He suspected that the symptoms described by Abelous and Billard were caused by infection. Pari observed bacilli in the frogs that showed the described symptoms, and by injecting these organisms into an unoperated frog he was able to produce the same symptoms that Abelous and Billard interpreted as a result of the extirpation of the thymus glands.

Hammar ('05), in a very thorough piece of work, was wholly unable to find any effects resulting from thymus extirpation when proper precautions were taken to guard against infection. It is seen that there is strong evidence against the results which Abelous and Billard attributed to thymus extirpation.

It might still be argued that the thymus glands may have an important function in regulating growth during larval life.

Adler ('14) gives the results of a series of experiments upon the larvae of Rana temporaria. The removal of the thymus glands was performed when the half-grown tadpoles had reached a length of 22.5 mm. to 23 mm. This was accomplished by means of an electric cautery. The operation caused a very high mortality. Twenty of the nine hundred tadpoles operated reached metamorphosis, but there was complete absence of the thymus glands in only three individuals and partial absence in five others. We thus find that his results are based upon a study of but three specimens. In the first place, he showed that the removal of the thymus glands in half-grown tadpoles does not prevent metamorphosis, nor is it accompanied by any external peculiarities. The gonads of these three specimens proved, however, to be decidedly larger than normal. In only one out of the three were they exceeded in length by any of the specimens in which the operation was unsuccessfully attempted. One unfortunate feature in Adler's account is the fact that he gives no statement regarding the sex of the specimens either thymusless or controls. It will be seen in my tables below that the ovaries of young frogs are normally much larger than the testes, at least in Rana pipiens. It is quite possible that his three thymusless specimens were all females. The fact that all of the controls had smaller germ glands than did two out of his three thymusless specimens could hardly be accepted as convincing evidence that the removal of the thymus glands has a marked influence in the development of gonads. This objection has especial force when we take into account the fact that Adler only measured the length of the germ glands and did not consider the other dimensions.

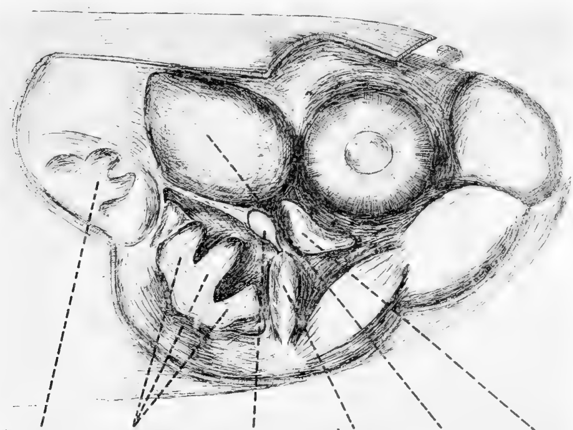
Adler finds that the thyroid glands in these three thymusless frogs are much larger than normal and that they are modified in texture. These points will be discussed below.

MATERIAL AND METHODS

Müller ('71), de Meuron ('86), and Maurer ('87) have given accounts of the origin of the thymus glands in the anurans. According to these authors, they arise as a pair of epithelial buds from the dorsal portion of the second gill pouches. This fact makes the anurans especially favorable for experiments in extirpation of these organs during larval life. In this series of experiments the larvae of Rana pipiens were used. The early thymus buds were removed with a pair of needles at the stage when the intestines were beginning to twist—8 to 8.5 mm. total length. One of the needles is broken off and ground to a knife edge. After narcotizing the tadpoles with chloretone, the knife-edged needle is inserted into the side of the head just beneath and behind the auditory vesicle and the cut is carried forward extending to a point beneath the eye. This usually causes considerable loss of blood, owing to the cutting of the anterior cardinal vein. There is a slight initial retardation of development probably resulting from this. But such effects are of short duration. Both needles are now used to spread open the wound. Any blood clots are removed, and the white spherical thymus bud is now seen as shown in the figure. The knife-edged needle is used to cut away this bud together with the adjacent portion of the second gill arch to which it is attached. Both needles must often be used to grasp and pick away the thymus bud. In each case the bud was carefully examined and identified after removal. Of course, it is necessary to operate on both sides in this manner. This necessitates making two separate incisions. The tadpoles rapidly recover and undergo normal development, passing through metamorphosis in the usual time and in normal fashion.

This experiment was performed in the spring of 1917 and 1918. The first season six of the operated tadpoles metamorphosed at the date indicated in the table. One other was somewhat undernourished and did not transform until the following spring when it was found to be of full size—rather above the ordinary. This, however, was the only case of retarded development and is in no wise different from similar cases observed among controls.

Four of these thymusless specimens were oedematous at the time of metamorphosis. Three died while the tail was fairly long, i.e., between 15 and 20 mm. in length. In all cases, however, the fore limbs were well developed and in none was the tail longer than the trunk. During the ensuing season the experiment was repeated, adding four cases of complete thymus removal and two cases in which it was incomplete. In none of these was there oedema nor was there marked mortality during metamorphosis



as in the season before. This is good evidence that these abnormalities were not due to the absence of the thymus glands. In fact, the evidence in favor of such an interpretation was slight enough on the basis of the 1917 experiments, one of the controls having shown the same degree of oedema.

The specimens were fixed in bichromate acetic. The utmost care was taken to search for the thymus glands or any possible remnants of them in the operated tadpoles. In five cases the entire head was sectioned as far back as the region of the fore limbs. The material having been previously stained in toto

with alum-cochineal was then cut into sections 25μ in thickness. These were searched most carefully for any evidence of thymus tissue, but no traces were found. In the other specimens the two sides of the head were cut away in such fashion as to include liberally the thymus region and parts near it. These were stained in toto, cleared, and examined in oil of wintergreen. Reliance could hardly be placed in this method, so they were later imbedded and cut into sections for further examination, every section being critically studied. By these laborious means it was possible to check up the accuracy of the operation in each specimen. It may be said with certainty that there were no vestiges ever so slight of the thymus glands in any of these cases. In nos. 12 and 13 only one thymus gland was in each case present, while in no. 14, a specimen that had been operated upon, both were found to be intact. Controls nos. 15 to 32 were reared under conditions like those of the thymusless specimens, while nos. 33 to 50 were purchased from a dealer. They had been preserved in formalin. Naturally, they are not as satisfactory as a check upon these results as are the other controls.

RESULTS

This work is most important in the fact that it is the first series of experiments in which the thymus glands were removed at their very inception. Experiments upon mature and even upon young animals lose much of their significance because they do not touch upon the important period of early development when the glands would be expected to exercise their greatest influence upon growth. While Adler ('14) operated upon earlier stages than had formerly been employed, the thymus glands had reached a high degree of development at the stage at which he operated, and had presumably been exerting their influence for some time, if they really function as endocrine glands at all. It is perfectly safe to conclude from my own experiments as well as from Adler's that the thymus glands exert no influence upon metamorphosis. It is also clear that they do not exert any appreciable influence upon growth in size. Attention has often been called to the interrelations in the mammals between the thymus glands

and the germ glands. The most striking facts in this connection are found in the persistence of the thymus glands after castration. This does not show that they in their turn exert any active influence upon the germ glands. In the review of literature attention has been called to the statement of Adler that there is a marked hypertrophy of the gonads in thymusless tadpoles and a criticism of his claims was offered. The conclusions drawn from the present work are entirely at variance with those of Adler. Owing to the fact that these tadpoles were reared under laboratory conditions, some of them were undersized. That is true of both the operated tadpoles and the controls. In comparing the gonads of operated and control frogs of equal size, it is impossible to demonstrate any consistent differences between them. For instance, compare the areas—length times breadth—of the male gonads of numbers 1, 12, and 15. Likewise numbers 5, 6, and 12. Also numbers 4, 6, and 20. As regards the female specimens, we must first of all eliminate number 11, because it did not metamorphose until a year after the operation. This is attributed to underfeeding under laboratory conditions, a feature that is illustrated by the small size of some of the specimens. There appeared to be a rhythm of tendency to metamorphosis that produced transformation in the spring although the tendency to metamorphosis is too weak in the fall to overcome the handicap of underfeeding. During the interval, the tadpole had continued to grow and the ovaries had likewise continued. This resulted in their attainment of unusual size at the time of metamorphosis—a fact evident not only in general dimensions, but in the dimensions of the oocytes. This independence between the germ gland growth and the general somatic differentiation had already been described in the case of thyroidless tadpoles (Allen, '17). Taking nos. 9 and 10, we find that general dimensions of ovaries and oocytes to be quite comparable to those of nos. 29 and 32, although both nos. 9 and 10 have fairly large ovaries. This taken in connection with the results in male specimens should be convincing. Adler asserts that the removal of the thymus glands causes a hypertrophy of the thyroid glands, at the same time producing changes involving a looseness of tex-

ture, increase in size and irregularity in form of follicles. He reports a deterioration in the quality of the colloid—a change to a more fluid condition showing an alveolar or fibrous character.

In my own study of the thyroid gland in thymusless tadpoles no abnormal features as to size or quality were demonstrated. The accompanying table requires some explanation. The heads of the thymusless frogs were cut in serial sections 25μ in thickness. The colloid was very tough—thoroughly normal in consistency and offered so much resistance to the knife that the glands were often torn where the knife encountered an especially thick colloid mass. The length of the thyroid gland in these series was estimated by counting the sections instead of by measuring as in controls nos. 21 to 37. In all the sectioned specimens the thyroid glands appeared to be abnormally short, although of normal width and with follicles of normal character. Since among the specimens thus sectioned, nos. 6 to 14, only nos. 12 and 14 were control frogs, we have insufficient means of comparing the operated and control frogs among the specimens thus treated. A comparison of these sectioned specimens with measured ones, nos. 1 and 3 and nos. 21 to 37, shows that the sectioned ones are almost invariably shorter than broad, while the reverse is true of the dissected specimens. It is quite probable that the toughness of the thyroid glands has caused them to be pulled out a trifle with each stroke against the knife. In this manner, cutting each time a thicker slice of the thyroid region than of the material as a whole. Tests showed that the microtome used is accurately constructed. By comparing the widths of the thyroid gland in the thymusless and control frogs, there seemed to be no consistent difference between them, and the difference in length favors the controls. The apparently greater size of the thyroid glands of the controls is probably due wholly to these differences in technique. If they have any significance at all, it is quite contrary to the findings of Adler.

CONCLUSIONS

1. The thymus glands from their very inception exert no influence upon growth or upon the progress of metamorphosis.

2. They are not at any stage of development indispensable to life, nor does their removal appear to cause any marked deficiency in the general metabolism of the body.

3. The extirpation of the thymus glands does not in any way modify the quality or the rate and degree of development of the gonads.

4. A rather incomplete study of the thyroid glands in the thymusless frogs fails to show that they were in any sense abnormal. It is fair to conclude that the thymus glands exert no influence upon the thyroid glands.

5. No modification of internal features was seen to result from the absence of the thymus glands.

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Table of measurements of the gonads

NUMBER	DATE KILLED	BODY LENGTH	TAIL LENGTH	GONADS						AVERAGE AREA	OOCYTES
				Right		Left		Area			
				Length	Breadth	Length	Breadth	Right	Left		
Thymusless											
		mm.	*mm.*	*mm*	*mm.*	*mm.*	*mm.*	*sq. mm.*	*sq. mm.*	*sq. mm.*	*mm.*
♂ 1	7/19	21.3	8.0	0.81	0.36	1.40	0.37	0.29	0.48	0.39	
♂ 2	7/26	20.4	3.0	1.12	0.41	0.87	0.43	0.46	0.37	0.42	
♂ 3	8/2	19.1	7.7	0.73	0.37	0.65	0.35	0.27	0.23	0.25	
♂ 4	8/5	21.3	14.8	1.03	0.46	1.25	0.46	0.47	0.57	0.52	
♂ 5	9/5	22.0	17.7	1.40	0.38	1.37	0.40	0.53	0.55	0.54	
♂ 6	7/15	22.2	0.0	0.86	0.40	0.92	0.36	0.34	0.33	0.34	
♂ 7	7/21	18.6	1.5	0.56	0.33	0.66	0.49	0.18	0.32	0.25	
♂ 8	7/21	17.1	1.5	0.59	0.40	0.63	0.30	0.24	0.19	0.22	
♀ 9	8/5	19.0	19.0	3.40	1.41	2.78	1.33	4.79	3.70	4.25	0.14 x 0.11
♀10	7/30	24.1	0.0	4.32	1.25	3.73	1.48	5.40	5.52	5.46	0.14 x 0.12
♀11	5/1	25.9	6.7	3.86	1.83	6.53	2.34	7.06	15.28	11.17	0.23 x 0.18
Partially successful operations											
♂12	7/25	21.3	2.0	1.02	0.42	1.48	0.42	0.43	0.62	0.43	
♀13	9/15	18.9	5.9	3.21	1.08	2.68	1.60	3.46	3.29	3.38	0.14 x 0.11
♀14	7/15	23.1	0.0	3.12	1.85	3.37	1.39	5.77	3.68	4.73	0.14 x 0.12
Controls											
♂15	7/28	19.8	0.0	0.85	0.56	1.06	0.45	0.48	0.48	0.48	
♂16	7/13	20.0	0.0	1.02	0.37	1.48	0.30	0.38	0.45	0.41	
♂17	7/28	20.1	0.0	1.35	0.36	1.65	0.33	0.49	0.54	0.52	
♂18	6/27	20.1	0.0	0.92	0.29	1.22	0.28	0.26	0.34	0.30	
♂19	6/30	20.9	0.0	0.99	0.34	1.09	0.34	0.34	0.37	0.35	
♂20	7/27	22.0	0.0	0.98	0.37	1.07	0.43	0.36	0.46	0.41	
♀21	7/16	22.2	5.4	1.06	0.31	1.29	0.29	3.25	3.78	3.52	
♀22	6/30	19.7	0.0	2.14	0.73	3.14	0.59	1.46	1.86	1.66	
♀23		20.8	0.0	4.88	1.22	4.36	1.35	5.96	5.88	5.92	
♀24	6/26	20.9	0.0	2.21	0.76	3.99	0.96	1.68	3.82	2.75	
♀25	6/26	20.9	0.0	3.63	0.86	2.97	1.09	3.11	3.23	3.17	
♀26	8/6	21.2	0.0	3.30	1.02	3.60	0.88	3.36	3.17	3.27	
♀27	7/16	21.7	0.0	3.13	0.89	4.06	1.35	2.79	5.49	4.14	
♀28	7/22	21.8	2.5	3.41	1.45	2.42	1.03	4.97	2.49	3.73	
♀29	7/27	22.2	0.0	3.43	1.62	3.31	1.82	5.56	6.02	5.49	0.12 x 0.11
♀30	6/23	22.5	0.0	3.07	1.06	3.23	0.96	3.24	3.09	3.17	
♀31	6/27	23.2	0.0	3.66	0.76	3.86	0.99	2.76	3.82	3.30	
♀32	6/26	24.1	0.0	3.66	1.32	3.79	1.29	4.83	4.88	4.86	

Table of measurements—Continued

NUMBER	DATE KILLED	BODY LENGTH	TAIL LENGTH	GONADS: Right, Length	Right, Breadth	Left, Length	Left, Breadth	Area, Right	Area, Left	AVERAGE AREA	OOCYTES
Purchased controls											
		mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*sq. mm.*	*sq. mm.*	*sq. mm.*	*mm.*
♂33		25.8	0.0	1.29	0.44	1.58	0.43	0.57	0.68	0.63	
♂34		25.3	1.6	1.53	0.36	1.48	0.30	0.55	0.44	0.50	
♂35		23.9	16.3	1.85	0.40	1.19	0.36	0.74	0.43	0.59	
♂36		24.9	13.1	1.91	0.43	2.47	0.40	0.82	0.99	0.91	
♂37		24.5	14.5	1.25	0.40	1.25	0.43	0.50	0.54	0.52	
♂38		25.4	2.0	1.45	0.40	1.88	0.33	0.50	0.62	0.56	
♂39		24.5	0.0	1.25	0.33	1.48	0.46	0.41	0.68	0.55	
♂40		23.3	2.3	0.92	0.36	1.16	0.43	0.33	0.50	0.44	
♂41		22.9	4.2	0.96	0.43	1.52	0.43	0.41	0.65	0.53	
♂42		27.2	12.9	1.48	0.36	1.69	0.30	0.54	0.51	0.53	
♂43		23.7	14.7	1.09	0.43	1.48	0.46	0.47	0.68	0.58	
♂44		23.2	0.0	1.78	0.26	1.68	0.30	0.46	0.50	0.48	
♂45		24.9	9.7	1.29	0.30	1.32	0.30	0.39	0.40	0.40	
♂46		25.2	13.4	1.02	0.35	1.31	0.33	0.36	0.43	0.40	
♀47		23.4	18.2	2.31	0.89	3.17	1.02	2.06	3.17	2.62	
♀48		22.6	3.1	2.34	1.12	3.50	1.09	2.62	3.81	3.22	
♀49		25.7	15.9	3.20	7.79	3.04	0.99	2.53	3.01	2.77	
♀50		22.7	12.6	3.53	0.83	2.98	0.86	2.93	2.98	2.96	

Dimensions of thyroid glands

NUMBER	DATE KILLED	BODY LENGTH	TAIL LENGTH	RIGHT THYROID: Length	Breadth	Area	LEFT THYROID: Length	Breadth	Area	AVERAGE AREA
		mm.	*mm.*	*mm.*	*mm.*	*sq. mm.*	*mm.*	*mm.*	*sq. mm.*	*mm.*
1	7/19	21.3	8.0	0.70	0.45	0.31	0.66	0.38	0.25	0.38
3	8/2	19.1	7.7	0.65	0.44	0.29	0.66	0.51	0.34	0.32
6	7/15	22.2	0.0	0.47	0.62	0.29	0.62	0.61	0.38	0.33
7	7/21	18.6	1.5	0.40	0.52	0.21	0.38	0.53	0.20	0.20
8	7/21	17.1	1.5	0.45	0.62	0.28	0.47	0.62	0.29	0.29
10	7/30	24.1	0.0	0.65	0.41	0.27	0.77	0.54	0.42	0.35
11	5/1	25.9	6.7	0.66	0.85	0.56	0.55	0.83	0.46	0.51
12	7/25	21.3	2.0	0.62	0.73	0.45	0.45	0.82	0.37	0.41
14	7/15	23.1	0.0	0.46	0.49	0.22	0.39	0.64	0.25	0.24
33		25.8	0.0	0.95	0.72	0.68	0.92	0.66	0.61	0.65
37		24.5	14.5	0.76	0.72	0.55	0.92	0.66	0.61	0.58
41		22.9	4.2	0.82	0.66	0.54	0.82	0.63	0.51	0.53
42		27.2	12.9	0.99	0.69	0.68	0.99	0.66	0.65	0.67
45		24.9	9.7	0.69	0.49	0.34	0.92	0.76	0.70	0.52
48		22.6	3.1	0.76	0.53	0.40	0.86	0.53	0.46	0.43
49		25.7	15.9	0.76	0.82	0.62	1.06	0.72	0.76	0.67

Resumen por el autor Bennet M. Allen.
Universidad de Kansas.

Las glándulas paratiroideas de las larvas de Bufo desprovistas de tiroídes.

Las glándulas tiroídes del renacuajo de Bufo fueron extirpadas por el autor sirviéndose de un método descrito previamente. La región de las glándulas tiroides y paratiroides fué cortada en serie y las estructuras de las últimas medidas por medio de un micrómetro ocular. La longitud, anchura y grosor de cada una de las cuatro glándulas fueron multiplicadas para obtener un valor que representa el volumen del paralelepípedo imaginario que contendría a la glándula. Los valores así obtenidos, en el caso de las cuatro paratiroides de un individuo determinado, fueron sumados para obtener un valor que representa groseramente el volumen total. Las conclusiones obtenidas son las siguientes: 1) La extirpación de las glándulas tiroides de Bufo causa una hipertrofia muy marcada de las glándulas paratiroides, que crecen sobrepasando varias veces el volumen de las glándulas tiroides de los ejemplares no operados. 2) No hay deposición de coloide en las glándulas paratiroideas de los renacuajos desprovistos de glándulas tiroides. Como resultado de la extirpación de las últimas no aparecen particularidades histológicas visibles en las glándulas paratiroides.

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THE PARATHYROID GLANDS OF THYROIDLESS BUFO LARVAE[1]

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It has been long known that there is a close interrelation between certain of the glands of internal secretion. Most of the earlier experiments upon the relationship between the thyroid and parathyroid glands have been carried out upon mammals, and for this purpose carnivores have been selected. In the removal of the thyroid gland the internal parathyroids have been likewise extirpated. The resulting enlargement of the lateral parathyroids in such cases might be attributed to the absence of the internal parathyroids, in part at least. The present work is free from this objection, because the operation of removing the thyroid gland from anuran larvae in no wise affects the parathyroids.

A brief discussion of the literature upon the relationship between these glands will be in order. Gley ('92, '95) discovered that the removal of the thyroid and inner parathyroid glands brought about hypertrophy of the lateral parathyroids. He also claimed that in these cases the parathyroids took on the structure of the thyroid glands and showed deposition of colloid material. In a later paper he retracted these statements regarding the assumption of the structural characteristics of the thyroid gland. Vincent and Jolly, also Vincent, Halpenny, and Thompson ('09) have reported on the development of thyroid characteristics in the lateral parathyroid glands of dogs after the removal of the thyroid and internal parathyroid glands. Biedl found that in the case of young dogs, removal of the thyroid glands

[1] A preliminary account of this work was presented at the 1918 meeting of the American Society of Zoölogists and published in the Anatomical Record, January 20, 1919. Press of work has prevented earlier publication.

caused a hypertrophy of the parathyroid glands to twice their normal size. He reports gland-like hollows containing colloid in the parathyroid glands of normal animals, but finds them to be far more numerous in cases where the thyroid gland has been removed. A number of writers have described a hypertrophy of the thyroid glands in cases where the parathyroid glands have been removed.

In the spring of 1918, the thyroid glands of Bufo were removed in the manner described in an earlier paper (Allen, '18). These tadpoles failed to metamorphose, as had been previously demonstrated. They were killed at various ages after having lived long beyond the period of normal metamorphosis and having reached gigantic size; they were for the most part, preserved in bichromate-acetic fluid. The more advanced young toads were fixed in 5 per cent formalin. The region of the thyroid and parathyroid glands was sectioned at a thickness of 10 μ, after staining in toto with alum cochineal. The controls used included specimens killed before, during, and after metamorphosis.

In the anurans the parathyroid bodies, four in number, are quite distinct from the thyroid glands and in close association with the aortic arches. They were measured by counting the number of sections through which each extended and by taking measurements in two dimensions upon the largest section of each. In each case an average of each dimension was taken for the four parathyroids of every specimen. The three average dimensions were then multiplied together to give the volume of a parallelopiped that would thus contain a parathyroid gland of average size. By such an approximation of volume it is possible to have a basis of comparison for the parathyroid glands of different specimens.

There is a large amount of variation in the average volume of the parathyroid glands in different specimens. It is difficult to account fully for this. While there are certain exceptional cases, as nos. 1, 6, and 11 among the controls and 26 among the thyroidless specimens, there is generally a certain amount of regularity in average size. While metamorphosis causes an appreciable diminution in body size and a decrease in the volume of

the thyroid, pituitary, and thymus glands (Allen, '18; Rogers, '18, and Larsen, '19), no such reduction in size is indicated in the present work. The number of specimens of this stage is too small to have any significance upon this point. In general there is a constant regular increase in the volume of the parathyroids of the controls. The number of nuclei counted in maximum-size sections shows the corresponding increase demonstrating that the growth in size is a genuine growth in the amount of tissue, not a mere loosening of the texture of the gland. Since counts of the nuclei had to be made in a section of each of the four parathyroid glands of each specimen, it is easy to see that the work of counting becomes very laborious when one is dealing with the large thyroidless tadpoles. Observations upon the other specimens in which counts were not made showed that the parathyroid glands were of equal density. For these reasons the count was not carried further. In these two cases where counts were made, there was such a large number of nuclei that the comparison with the older metamorphosed controls was perfectly clear. It is quite safe to say that the relative density of the parathyroid glands of thyroidless tadpoles is equal to that of the parathyroids of the controls and that their much larger size in the thyroidless tadpoles probably indicates that in these they have a greater functional importance than in the controls. Even though the average body length of the young Bufo controls, studied in this work, was 50 per cent greater than the body length of the thyroidless specimens, their parathyroid glands were much less than one-fourth as large as those of the thyroidless tadpoles. In comparing the large thyroidless tadpoles with the young control tadpoles in the corresponding state of differentiation, nos. 1 to 5, it is seen that the parathyroid glands of the controls have less than one-fifteenth the volume of the thyroidless specimens. While the average body length of these controls is as 10.2 mm. compared with 17.9 mm.

In order to give a more accurate criterion for estimating the relative average volume of the parathyroid glands of specimens of different size and stage of development, the cube root of the general average volume in each group is given. This would give

a linear dimension of each volume value which may be roughly compared with the average length measurement of the group considered. While no claim is made for mathematical refinement, especially in view of the marked change of form due to metamorphosis, nevertheless the writer feels that a fair comparison may be made from the data at hand and that the results are sufficiently striking to justify the conclusions drawn. These figures speak for themselves and no further comment seems necessary.

There were no evidences of colloid deposition in the parathyroids of thyroidless tadpoles, even though the thyroid glands of tadpoles in corresponding stage of development have a large amount of colloid, and though they have reached an age and a size corresponding to those of young toads long after metamorphosis—a stage when colloid is normally present in the thyroid glands in large amount. This shows quite clearly that in the toad at least the parathyroid glands do not vicariously assume the function of colloid production after removal of the thyroid glands, as asserted by the authors mentioned in the discussion of literature. There are, in fact, no peculiarities of any kind recognizable in the structure of the parathyroid glands of thyroidless tadpoles.

CONCLUSIONS

1. Removal of the thyroid glands of Bufo causes a very marked hypertrophy of parathyroid glands, so that they grow to many times the volume attained by the thyroid glands of unoperated specimens.

2. There is no deposition of colloid in the parathyroid glands of thyroidless tadpoles nor any other evidence of the assumption of a vicarious relationship between the thyroid and parathyroid glands. There are no noticeable histological peculiarities of the parathyroid glands resulting from the removal of the thyroid glands.

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Table of detailed measurements of parathyroid glands

NUMBER		RIGHT		LEFT		AVERAGE	VOLUME
		Anterior	Posterior	Anterior	Posterior		
1	Total length, 15.1 mm. Body length, 8.4 mm.						
		mm.	*mm.*	*mm.*	*mm.*	*mm.*	*cmm.*
	L.	0.100	0.080	0.090	0.070	0.085	
	W.	0.088	0.071	0.086		0.079	
	T.	0.057	0.050	0.067		0.054	0.00036
	No. nuclei	92	72	119	74	89	
2	Total length, 20.8 mm. Body length, 9.5 mm.						
	L.	0.070	0.070	0.060	0.050	0.062	
	W.	0.043	0.063	0.070	0.053	0.057	
	T.	0.064	0.043	0.043	0.031	0.044	0.00015
	No. nuclei	83	89	54	61	72	
3	Total length, 25.9 mm. Body length, 10.9 mm.						
	L.	0.070	0.060	0.080	0.070	0.070	
	W.	0.090	0.067	0.071	0.066	0.073	
	T.	0.050	0.043	0.049	0.036	0.044	0.00022
4	Total length, 25.2. Body length, 11.6 mm.						
	L.	0.070	0.060	0.060	0.060	0.062	
	W.	0.058	0.053	0.069	0.058	0.060	
	T.	0.043	0.045	0.043	0.050	0.046	0.00017
5	Total length, 20.8. Body length, 10.6 mm.						
	L.	0.070	0.050	0.080	0.060	0.065	
	W.	0.058	0.080	0.088	0.053	0.074	
	T.	0.044	0.045	0.034	0.038	0.040	0.00019
	No. nuclei	89	79	75	65	77	
6	Total length, 24.8. Body length, 11.4 mm.						
	L.	0.070	0.080	0.090	0.070	0.077	
	W.	0.094	0.139	0.100	0.066	0.099	
	T.	0.073	0.054	0.061	0.061	0.061	0.00046
	No. nuclei	153	169	147	95	111	
7	Total length, 13.1. Body length, 10.1 mm.						
	L.	0.070	0.070	0.080	0.080	0.075	
	W.	0.067	0.057	0.060	0.059	0.060	
	T.	0.049	0.041	0.053	0.047	0.047	0.00021
	No. nuclei	75	60	88	65	72	

Table of detailed measurements of parathyroid glands—Continued

NUMBER		RIGHT Anterior	RIGHT Posterior	LEFT Anterior	LEFT Posterior	AVERAGE	VOLUME
8	Total length, 12 mm. Body length, 12 mm.						
		mm.	*mm.*	*mm.*	*mm.*	*mm.*	*cmm.*
	L.	0.070	0.070	0.070	0.080	0.072	
	W.	0.061	0.044	0.057	0.071	0.058	
	T.	0.050	0.039	0.053	0.041	0.046	0.00019
	No. nuclei	75	68	91	68	76	
9	Body length, 11.3 mm.						
	L.	0.080	0.050	0.060	0.060		
	W.	0.066	0.051	0.059	0.041		
	T.	0.054	0.039	0.049	0.041		0.000167
10	Body length, 11.1 mm.						
	L.	0.060	0.060	0.070	0.050		
	W.	0.060	0.050	0.073	0.046		
	T.	0.046	0.033	0.046	0.037		0.000144
11	Body length, 11.1 mm.						
	L.	0.070	0.050	0.090	0.050		
	W.	0.073	0.064	0.051	0.041		
	T.	0.049	0.036	0.051	0.041		0.000183
12	Body length, 10.4 mm.						
	L.	0.070	0.060	0.070	0.050		
	W.	0.064	0.047	0.054	0.047		
	T.	0.057	0.043	0.050	0.037		0.000181
	Young toads (long after metamorphosis)						
13	Body length, 21.7 mm.						
	L.	0.090	0.100	0.100	0.090	0.095	
	W.	0.094	0.105	0.113	0.108	0.106	
	T.	0.053	0.066	0.059	0.044	0.055	0.00055
	No. nuclei	1.56	158	205	166	171	
14	Body length, 23.8 mm.						
	L.	0.160	0.100	0.140	0.100	0.125	
	W.	0.123	0.086	0.114	0.114	0.109	
	T.	0.073	0.060	0.077	0.060	0.067	0.00091

Table of detailed measurements of parathyroid glands—Continued

NUMBER		RIGHT		LEFT		AVERAGE	VOLUME
		Anterior	Posterior	Anterior	Posterior		
15	Body length, 24.2 mm.						
		mm.	*mm.*	*mm.*	*mm.*	*mm.*	*cmm.*
	L.	0.130	0.120	0.110	0.170	0.132	
	W.	0.141	0.111	0.123	0.103	0.121	
	T.	0.100	0.079	0.107	0.073	0.090	0.00143
16	Body length, 24.4 mm.						
	L.	0.120	0.130	0.140	0.100	0.117	
	W.	0.126	0.122	0.111	0.087	0.111	
	T.	0.082	0.082	0.080	0.059	0.074	0.00096
17	Body length, 27.0 mm.						
	L.	0.221	0.070	0.200	0.110	0.150	
	W.	0.159	0.063	0.131	0.133	0.121	
	T.	0.076	0.036	0.078	0.055	0.057	0.00103
	Giant thyroidless tadpoles						
18	Total length, 37.5 mm. Body length, 14.1 mm.						
	L.	0.170	0.170	0.110	0.180	0.157	
	W.	0.167	0.149	0.171	0.300	0.196	
	T.	0.151	0.113	0.111	0.116	0.123	0.00379
	No. nuclei	403	247	294	304	312	
19	Total length, 42 mm. Body length, 16.3 mm.						
	L.	0.130	0.140	0.140	0.260	0.167	
	W.	0.140	0.146	0.157	0.137	0.144	
	T.	0.088	0.103	0.103	0.060	0.089	0.00215
20	Total length, 42 mm. Body length, 16.4 mm.						
	L.	0.170	0.160	0.160	0.130	0.155	
	W.	0.136	0.123	0.222	0.250	0.182	
	T.	0.093	0.059	0.069	0.047	0.065	0.00183
21	Total length, 40.1 mm. Body length, 17 mm.						
	L.	0.190	0.150	0.150		0.163	
	W.	0.169	0.193	0.140		0.167	
	T.	0.114	0.101	0.147		0.120	0.00326
	No. nuclei	282	272	263		272	

Table of detailed measurements of parathyroid—Concluded

NUMBER		RIGHT		LEFT		AVERAGE	VOLUME
		Anterior	Posterior	Anterior	Posterior		
22	Total length, 46 mm. Body length, 17.8 mm.						
		mm.	*mm.*	*mm.*	*mm.*	*mm.*	*cmm.*
	L............	0.170	0.180	0.230	0.170	0.187	
	W............	0.207	0.157	0.162	0.117	0.160	
	T............	0.129	0.079	0.107	0.092	0.101	0.00302
23	Total length, 49 mm. Body length, 18.3 mm.						
	L............	0.130	0.160	0.150	0.180	0.155	
	W............	0.107	0.183	0.167	0.154	0.150	
	T............	0.057	0.083	0.126	0.104	0.093	0.0218
24	Total length, 43.7 mm. Body length, 19.6 mm.						
	L............	0.200	0.160	0.250	0.200	0.202	
	W............	0.179	0.176	0.195	0.157	0.177	
	T............	0.140	0.114	0.097	0.093	0.110	0.00393
25	Total length, 47.1 mm. Body length, 20.4 mm.						
	L............	0.160	0.130	0.220	0.170	0.170	
	W............	0.169	0.126	0.223	0.157	0.169	
	T............	0.146	0.083	0.167	0.136	0.133	0.00382
26	Total length, 49.6 mm. Body length, 20.7 mm.						
	L............	0.270	0.230	0.180	0.270	0.238	
	W............	0.233	0.269	0.176	0.256	0.233	
	T............	0.129	0.083	0.133	0.114	0.114	0.00632

NUMBER	TOTAL LENGTH	BODY LENGTH	AVERAGE VOLUME OF PARATHYROID GLANDS	$\sqrt[3]{\ }$ OF AVERAGE VOLUME OF PARATHYROID GLANDS	AVERAGE NUMBER OF NUCLEI IN PARATHYROID GLANDS
		Unmetamorphosed controls			
	mm.	*mm.*	*cmm.*	*mm.*	
1	15.1	8.4	0.00036		89
2	20.8	9.5	0.00015		73
3	25.8	10.9	0.00022		
4	26.2	11.6	0.00017		
5	20.8	10.6	0.00019		77
Average		10.2	0.00022	0.060	80
		Metamorphosing controls (forelimbs through)			
6	24.8	11.4	0.00046		141
7	23.2	10.1	0.00021		72
8		12.0	0.00019		76
Average		10.8	0.00029	0.066	96
		Metamorphosed controls			
9		11.3	0.00017		
10		11.1	0.00014		
11		11.1	0.00018		
12		10.4	0.00018		
Average......		11.0	0.00017	0.855	
		Young Bufo—long after metamorphosis			
13		21.7	0.00055		143
14		23.8	0.00091		
15		24.2	0.00143		
16		24.4	0.00096		
17		27.0	0.00103		
Average......		24.2	0.00098	0.099	143
		Thyroidless tadpoles			
18	37.5	14.1	0.00379		312
19	42.0	16.3	0.00215		
20	42.0	16.4	0.00183		
21	40.1	17.0	0.00326		272
22	46.0	17.9	0.00302		
23	49.3	18.3	0.00218		
24	43.7	19.6	0.00393		
25	47.1	20.4	0.00382		
26[1]	49.6	20.7	0.00632		
Average		17.9	0.00337	.150.50	292

[1] In No. 26 the parathyroid glands were heart-shaped, hence their actual volume would not be so great as indicated.

Resumen por el autor, L. V. Heilbrunn.
Universidad de Michigan.

Estudio experimental de la división celular.

La viscosidad del citoplasma de los óvulos de erizo de mar en vias de división puede comprobarse por el método de la centrifugación. En relación con la mitosis tienen lugar cambios marcados de viscosidad; a un aumento inicial de viscosidad sigue una disminución de la misma. El considerable aumento de viscosidad puede expresarse como una gelificación, que alcanza su máximo precisamente antes de la aparición del huso. Los cambios de viscosidad determinan aparentemente la aparición del anfiaster o huso. Cuando se suprime la gelificación, no se forma figura mitósica, aun cuando los óvulos pueden permanecer ilesos. Tal supresión de la formación de la gelatina fué producida por el autor usando varios anestésicos y tambien el frío. Aunque su efecto final es el mismo, el frío y el éter son mutuamente antagónicos. Las soluciones hipertónicas aumentan la viscosidad del citoplasma de los óvulos en vías de division, y tienden a suprimir la reversión normal de la gelificación. El cianuro potásico y la cloretona producen un efecto semejante. Existen algunas pruebas en favor de la idea que considera a la gelificación mitósica como el producto de la extración de agua.

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AN EXPERIMENTAL STUDY OF CELL-DIVISION

I. THE PHYSICAL CONDITIONS WHICH DETERMINE THE APPEARANCE OF THE SPINDLE IN SEA-URCHIN EGGS

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INTRODUCTION

In my work on artificial parthenogenesis, I showed that all substances which incite the sea-urchin egg to divide mitotically produce a marked increase in the viscosity of the cytoplasm. A similar viscosity increase was also found to occur after the normal stimulus of fertilization. Accordingly, I held the view that some sort of 'solidification' was the essential factor which initiated mitosis. For purposes of clearness, I shall refer to this stiffening as a gelation. In order to prove my view correct, I have now attempted to show that if this gelation is prevented, no stimulus to mitosis can occur. If the cytoplasm of a fertilized egg is kept in the fluid condition, then no mitotic figure forms, and the egg remains undivided, although it is not necessarily injured by the treatment. Here, then, is additional evidence in favor of the view that the formation of the amphiaster is a direct consequence of cytoplasmic gelation.

It can readily be seen that such a study of mitosis leads in two directions. In the first place, it throws light on the genesis of the mitotic figure; secondly, it affords a physical interpretation of stimulation and anesthesia in the egg. In this contribution I propose to emphasize the first of these problems, reserving the discussion of anesthesia for a later paper.

The experimental work which furnishes the basis for this paper was done at Woods Hole during the summer of 1916. The form used was Arbacia punctulata (Gray).

VISCOSITY CHANGES DURING MITOSIS

During the process of mitosis the cytoplasm of the sea-urchin egg undergoes marked physical changes. I have already shown (Heilbrunn, '15) that a gelation occurs soon after mitosis, so that the early stages of the mitotic process are associated with a gradual stiffening of the egg protoplasm. In later stages this gelation is reversed, so that in the anaphase the egg has again returned to its original fluid condition.

In order to follow closely the physical changes which occur in the cytoplasm, viscosity measurements were made by the centrifuge method. An accurate measure of the cytoplasmic viscosity can be obtained by a determination of the ease with which granules move through it. When the cytoplasm is quite fluid, a moderate centrifugal force is sufficient to throw all the granules into one half of the egg, so that a clear 'hyaline zone' extends through the other half (except for a small polar accumulation of lipoids, the 'gray cap'). As the viscosity of the cytoplasm increases, the granules cannot respond so readily to centrifugal force, and the hyaline zone, instead of extending through half of the egg, only appears in a limited portion. With still greater increase in viscosity, the same or even a stronger centrifugal force causes no perceptible movement of the granules. The width of the hyaline zone is not so easy to determine in the fertilized egg as in the unfertilized egg, for soon after fertilization the pigment granules characteristic of the Arbacia egg all migrate to the cortex, and since they are not so readily moved when in this position, they remain as an outer coat completely surrounding and partially obscuring the interior cytoplasm. But with a little practice this difficulty is easily overcome by proper focusing, and it becomes a simple matter to estimate the width of the hyaline zone. So great are the changes in its extent that it is sufficient to express its width approximately in terms of the egg diameter. In this way rapid determinations can be made and viscosity tests can be completed in a very short time. Thus, in a number of experiments I was able to determine the egg viscosity at intervals of about four minutes.

The centrifuge used was a Bausch & Lomb hand centrifuge, equipped with two speeds. In the following experiments, however, the high speed was always employed. This necessitated a use of the hematocrit attachment, the eggs being placed in small glass tubes. Because of the difficulty with which such small tubes are cleaned, it was found advisable to use new tubes for each centrifuge test. A pair of tubes can be made in less time than it takes to thoroughly cleanse them.

In comparing the viscosity of eggs at one stage with the viscosity at a later stage, two methods of procedure are possible. In the first place, the eggs can be fertilized at two different times, and then eggs from both lots centrifuged simultaneously. In this way, the experimenter can assure the same treatment to both sets of eggs. Earlier tests were made in this way, and it was possible to demonstrate that after fertilization the egg viscosity first increased and then decreased. But a closer study of the process demanded viscosity tests at frequent intervals. In order to accomplish this conveniently, only a single lot of eggs can be studied. The experimenter must therefore learn to handle the centrifuge with such precision that the centrifugal force generated in each of the tests is constant. The handle of the centrifuge has to be turned a certain number of times in a certain number of seconds, and great care must be taken that the turning is uniform, for the centrifugal force of course depends on the speed. With a little practice, one may acquire the knack of turning the handle with machine-like precision.

In the accompanying tables, the amount of centrifugal force exerted on the eggs is given precisely by recording the number of times the handle was turned in a certain time interval. Each turn of the handle resulted in 130 revolutions of the tubes. The distance between the ends of the tubes was 12 cm. The centrifugal force is given by the formula $C = \frac{mv^2}{r}$. In this formula, the mass m is unknown, but presumably constant, the velocity $v = 2\pi r \times$ the number of turns per second, r the radius is always 6. Thus, when the handle is turned at different speeds, the amount of centrifugal force generated varies directly as the square of the number of turns per second.

In order to make the tables as brief and compact as possible, many less important observations have been omitted. In the third column, in indicating the number of times the centrifuge handle was turned and the number of seconds consumed in the process, the number of turns is given first and this is followed by the number of seconds. Thus "30 in 25" indicates that the handle was turned thirty times in twenty-five seconds. In each case the turning is assumed to have been uniform unless otherwise stated. In the fourth column, the extent of the hyaline zone, and, in many cases, the degree of distinctness with which it appeared, is recorded. In referring to the hyaline zone, the abbreviation 'H.Z.' is sometimes employed; more generally, however, it is omitted, the remarks being then understood to pertain to the hyaline zone. The various fractions in the fourth column are indices of the extent of the hyaline zone. A line passing perpendicularly to the zones of the centrifuged eggs and through the egg center is regarded as the axis of stratification. The fraction of this line which is included by the hyaline zone is taken as an index of the extent of this zone.

The temperature of the water containing eggs was recorded both at the beginning and at the end of each experiment. As every one knows, the length of time necessary for cleavage varies with the temperature. In the second column is recorded the time when cleavage began. Of course, there is always some variation among the eggs of a given batch. Usually a very few eggs start ahead of the others. A minute or two later, 5 to 10 per cent of the eggs can be observed segmenting, and in another minute about 40 per cent will be dividing. The cleavage time indicated in the tables is the time when about 5 to 10 per cent of the eggs have begun to divide.

In order to correlate the viscosity tests with the mitotic changes occurring within the egg during the tests, the eggs were observed as closely as possible. The Arbacia egg is unfortunately rather opaque, and comparatively little cytological detail can be observed in the living egg. After fertilization, the first indication of mitosis is a long, clear streak of hyaline material. This streak is probably the profile view of the disc which Fol described in 1877,

and named the 'amas sarcodique.' It later becomes converted into the spindle, its end forming the material for the asters. I shall, for convenience, refer to it as the 'pre-spindle.'

Of course, with centrifuge tests every four minutes, it was impossible to keep the eggs under constant observation. Accordingly, I could not record the exact minute when the pre-spindle first appeared. Nevertheless, in some of the experiments, I was able to fix the time of its appearance within narrow limits. And this is all that could be hoped for, anyway.

These observations and measurements of the living egg might well be supplemented by observations and measurements of eggs fixed after being centrifuged. I hope to make such a study at some time in the future.

I pass now to the consideration of individual experiments:

Eggs fertilized at 10:35 A.M.

Temperature { 22.3° at 10:35 A.M. / 23.3° at 11:30 A.M.

MINUTES AFTER FERTILIZATION	OBSERVATIONS ON NORMAL (i.e., UNCENTRIFUGED) EGGS	CENTRIFUGAL TREATMENT	HYALINE ZONE
6		50 in 30	½
10		50 in 30	½ not distinct
14		50 in 29	H.Z. not sharply marked off
19	No spindles	50 in 30	H.Z. indicated toward lighter pole, but not very distinctly.
24⅓		50 in 31	Barely indicated, at most ⅕ to ¼
26½	No spindles		
27½	Pre-spindles beginning to appear		
28⅓		50 in 30	¼ to ⅓
33		50 in 31	½ shows plainly
37		50 in 31	½ shows plainly
43		50 in 31	½ shows plainly
46	Cleavage beginning		

September 2nd

Eggs fertilized at 10:20 A.M.

Temperature {21.0° at 10:20 A.M. / 21.8° at 10:28 A.M.}

MINUTES AFTER FERTILIZATION	OBSERVATIONS ON NORMAL (i.e., UNCENTRIFUGED) EGGS	CENTRIFUGAL TREATMENT	HYALINE ZONE
6		50 in 30	½
10		50 in 30	In some eggs, H.Z. shows plainly and extends ½ way, in other eggs, it is indistinct
14		50 in 31	Usually does not show, in some eggs, ⅙
18	No spindles		
20		50 in 32	¼ in eggs best stratified.
22	Pre-spindles first noted		
24		50 in 31	¼ to ⅓ in best eggs
28		50 in 32	⅓ in best eggs
32		50 in 31	⅓ to ½
36		50 in 31	½
40		50 in 31	½
45		50 in 31	½
48	Cleavage beginning about 10 per cent		

September 4th

Eggs fertilized at 11:55 A.M.

Temperature {31.0° at 11:54 A.M. / 22.8° at 12:47 P.M.}

6		30 in 22	½ distinct
10		30 in 22½	H.Z. indistinct toward lighter pole
14		30 in 22½	No trace of a H.Z.
18		30 in 22	No trace of a H.Z.
19	No spindles		
21	Pre-spindles show plainly		
22		30 in 23	In a few eggs, H.Z. may be indicated, but this is doubtful
26		30 in 22	⅓ in a few eggs
30		30 in 22	⅓ to ½, but not very distinct
34		30 in 22½	Nearly ½, but it does not show very plainly
38		30 in 22½	⅓ to ½, shows more plainly than in previous test
42		30 in 22	½, shows very plainly
46		30 in 23	Most eggs show no H.Z. In some, however, ⅓ to ½.
48½	About 5 per cent segmenting		

September 6th

Eggs fertilized at 11:50 A.M.
Temperature: 19.8° at 11:50 A.M.; 21.0° at 12:44 P.M.

MINUTES AFTER FERTILIZATION	OBSERVATIONS ON NORMAL (i.e., UNCENTRIFUGED) EGGS	CENTRIFUGAL TREATMENT	HYALINE ZONE
2		30 in 22	$\frac{1}{3}$
7		30 in 23	$\frac{1}{3}$ to nearly $\frac{1}{2}$, eggs appear to be better stratified than in previous test
11		30 in $23\frac{1}{2}$	Usually H.Z. does not show. Rarely $\frac{1}{3}$
15		30 in 22	No H.Z.
18	No spindles		
19		30 in 22	No H.Z.
20	No spindles		
$21\frac{1}{2}$	Pre-spindles present		
23		30 in 22 (turns not quite regular)	Faintly indicated in a few eggs
27		30 in 23	Barely indicated in a few eggs
31		30 in 22	$\frac{1}{2}$, but not all distinct
35		30 in 22	$\frac{1}{2}$, but not very distinct
39		30 in 22	$\frac{1}{2}$, much more distinct
45		30 in 22	$\frac{1}{2}$, shows plainly
51	About 10 per cent segmenting		

September 9th

Egg fertilized at 1:45 P.M.

Temperature {19.8° at 1:46 P.M. / 20.8° at 3:50 P.M.}

MINUTES AFTER FERTILIZATION	OBSERVATIONS ON NORMAL (i. e., UNCENTRIFUGED) EGGS	CENTRIFUGAL TREATMENT	HYALINE ZONE
4		30 in 25	$\frac{1}{3}$
9		30 in 25	$\frac{1}{2}$
13		30 in 25	Usually does not show, rarely as much as $\frac{1}{3}$
18		30 in 25	No H.Z.
22	No spindles		
23		30 in 25	No H.Z.
26	Pre-spindles present		
28		30 in 25	No H.Z.
32		30 in 25	Faintly indicated in some eggs
36		30 in 25	H.Z. indicated toward one pole
40		30 in 26	$\frac{1}{3}$ or slightly more
$44\frac{1}{2}$		20 in 20	$\frac{1}{3}$
51	See 4th column	30 in 25	$\frac{1}{3}$. Some eggs show cleavage furrows beginning
55		30 in 25	No eggs show H.Z. or any trace of stratification
59		30 in 24	No H.Z.
63		30 in 24	No H.Z.
67		30 in 24	Indicated in a few eggs
71		30 in 25	Indicated in some eggs
76		30 in 25	Extends as much as $\frac{1}{3}$ way
82		30 in 25	Nearly $\frac{1}{2}$
$86\frac{1}{2}$		30 in 25	In eggs still in 2-cell stage H.Z. shows, but those which are undergoing the 2nd cleavage do not show it

September 11th

Eggs fertilized at 4:40 P.M.

Temperature {21.0° at 4:30 P.M. / 18.6° at 6:02 P.M.}

MINUTES AFTER FERTILIZATION	OBSERVATIONS ON NORMAL (i.e., UNCENTRIFUGED) EGGS	CENTRIFUGAL TREATMENT	HYALINE ZONE
3		30 in 25	$\frac{1}{3}$
7		30 in $25\frac{1}{2}$	$\frac{1}{4}$
12		30 in 25	No H.Z.
16		30 in 25	No H.Z.
$19\frac{1}{2}$	No spindles		
20		30 in 23	H.Z. indicated by a light spot at one pole. (Nucleus?)
21	No spindles		
23	Pre-spindles present		
24		30 in 25	H.Z. indicated at one pole
28		30 in 25	$\frac{1}{5}$ to $\frac{1}{4}$
32		30 in 25	$\frac{1}{3}$, usually indistinct
36		30 in 25	$\frac{1}{3}$, fairly distinct
40		30 in 25	$\frac{1}{3}$ to nearly $\frac{1}{2}$, distinct
44		30 in 25	$\frac{1}{3}$ to nearly $\frac{1}{2}$, distinct
47		30 in 25	$\frac{1}{3}$ to $\frac{1}{2}$, indistinct
50		30 in 25	$\frac{1}{3}$ to $\frac{1}{2}$, indistinct
$52\frac{1}{6}$		30 in 25	$\frac{1}{3}$ to $\frac{1}{2}$, indistinct
55		30 in 25	H.Z. appears indistinctly and consists largely of the wide anaphase spindle
59	About 5 per cent segmenting		
61		40 in 30	No H.Z.
65		40 in 30	No H.Z. except in a few cases when one end of cells is slightly paler than the opposite end
69		40 in 30	When the axis of stratification is parallel to the 1st cleavage plane, the H.Z. shows indistinctly $\frac{1}{3}$ way.
73		40 in 30	When the axis of stratification is parallel to the 1st cleavage plane, the H.Z. extends nearly $\frac{1}{2}$ way. It also shows readily in other positions
78		40 in 30	Same as in previous test
95	About 5 to 10 per cent have undergone second cleavage		

All of the above tables tell the same story. During the mitotic process there is a marked increase in viscosity, which is later followed by a decrease. These changes in viscosity are very considerable. Soon after fertilization and again before the first cleavage, a hyaline zone can be made to appear distinctly through a large part of the egg, if the centrifuge handle is turned thirty times in twenty-five seconds. On the other hand, at about twenty minutes after fertilization, even if the centrifuge handle is turned fifty times in thirty seconds, the hyaline zone either does not appear at all or shows in only a small region of the egg. In the latter case the force exerted is approximately twice as great. The increase in viscosity is at least twofold and is almost certainly far more than this. Such a marked viscosity increase is beyond much doubt due to a gelation with the cytoplasm.

As the tables show the gelation reaches its height slightly prior to the time that the pre-spindle first becomes visible. The appearance of the spindle is then followed by a decrease in viscosity. These facts suggest a definite time relation between the viscosity changes and the formation of the spindle.

During the second mitotic division of the egg, there is a parallel series of viscosity changes. As the first cleavage occurs, and apparently even before the cells are fully separated, there is a renewed stiffening of the cytoplasm. This is in preparation for the second mitosis. This gelation lasts ten or fifteen minutes, and then the cytoplasm becomes more fluid again. No attempt was made to follow similar changes in later divisions of the egg.

The fact that gelation occurs during mitosis is indicated by various other observations. In his book on the cell, Flemming pointed out that during the process of division the refractive index of the cell rises. With this fact he correlated the increased staining capacity of fixed preparations of dividing cells, as first observed by van Beneden ('75). Flemming ('82) apparently believed that these observations indicated a stiffening of the cytoplasm. In a later paper ('91) he states that the refractive index of a dividing connective-tissue cell increases, and then in the anaphase begins to decrease again. Similar observations have been made by Levi ('16), apparently without any knowledge of Flemming's work.

An observation of Spek ('18) on Nematode eggs is also of interest. In these eggs, as is well known, the cytoplasm near the surface is in continual movement. But at the moment when the spindle becomes visible all this movement ceases completely. Spek finds "alle amöboide Bewegungen an der Oberfläche der Eizelle . . . . ganz plötzlich—in dem Augenblick, wo der Spindel sichtbar wird—völlig aufhören." Probably this cessation of movement is due to a cytoplasmic stiffening. This stiffening, however, does not extend to the center of the egg, for in the central region the spindle oscillates from side to side.

In sea-urchin eggs Albrecht ('98) found that there was an increase in viscosity after fertilization. This was more conclusively shown by myself in 1915.

But the most remarkable work is that of Chambers ('17). By microdissection he was able to demonstrate in the dividing eggs first a gelation and later a return to a more fluid condition. My findings agree absolutely with his. Moreover, Chambers was able to study the morphological aspect of the gelation. In the astral radiations only the granular material is solidified. The hyaline rays which lie between the granular radiations are fluid.

The solidified materials of the cytoplasm apparently extend out to the cortex of the egg and are attached there. This is indicated by the shape of the egg in the centrifuge tests. When they are centrifuged soon after fertilization, the contour of the egg remains spherical. However, as soon as gelation begins, the eggs assume irregular shapes after centrifugal treatment. This distortion of the eggs always occurs at this time and is quite striking. Oftentimes various regions of the eggs are flattened, and frequently parts of the egg surface are indented, indicating a strong pull on certain regions. In the later stages of mitosis, when the cytoplasm is again more fluid, the eggs nevertheless assume irregular shapes when centrifuged. Apparently, there are still some gelatinous strands, perhaps more slender, which retain their connection to the egg surface, and pull upon it when the egg is centrifuged. This view is supported by the fact that when such eggs are centrifuged more vigorously, there is less tendency for irregularity in contour. In this case, very probably

the strands are torn from their attachment and the shape of the egg can remain spherical.

The conception of astral radiations or gelatinous strands of some kind attached to the periphery of the cell is of importance in any theoretical interpretation of cell division. That there is actually such an attachment is also indicated by a direct observation of the egg during the mitotic process. On a number of occasions I have noticed that the first appearance of the amphiaster in the egg is followed by a change in the outline of the surface contour. The hyaline membrane, at first perfectly smooth, now appears slightly crenate. This crenate appearance is more evident when the egg is looked at from one pole of the mitotic spindle, and is apparently due to the pull of gelatinous strands which radiate from the centrosphere. I have not been able to observe such a crenate appearance in all eggs.

INHIBITION OF SPINDLE FORMATION

The measurements of cytoplasmic viscosity recorded in the previous section afford evidence that the appearance of the mitotic figure is closely bound up with gelation phenomena. Much more convincing evidence can be obtained experimentally. My earlier work showed that artificial stimulation to mitosis, no matter what the reagent, always involves a preliminary gelation of the cytoplasm. These observations indicated that there is a definite causal sequence, but there was nothing to show that gelation was more than a secondary phenomenon, having no direct relation to the formation of the mitotic figure. In order to show that the appearance of the spindle depends absolutely on a preliminary cytoplasmic gelation, it is necessary to show that suppression of the gelation in every case results in a suppression of the spindle. This I have been able to do.

In searching for an antigelatinizing agent, I was guided by the earlier observations of Hertwig ('90) and Wilson ('01). The former discovered that cold effectively prevented the appearance of the amphiaster, the latter found that ether acted in a similar manner.

In the course of my work I have been able to discover a large number of substances which prevent the normal gelation of the egg or reverse it after it has occurred.

If the fertilized eggs are treated with antigelatinizing agents before the spindle has appeared, spindle formation is inhibited and cell division does not occur. This suppression of cell division need not involve injury to the egg, and in most cases when the eggs are returned to normal sea-water they resume their development.

The following substances were found, in proper concentration, to prevent gelation: ether, chloroform, acetone, paraldehyde, propyl alcohol, isoamyl alcohol, ethyl butyrate, ethyl nitrate, acetonitrile, nitromethane, chloral hydrate, phenyl and ethyl urethanes.

In studying the effect of these substances on the egg, the usual procedure was as follows: Soon after fertilization, eggs were placed in a series of concentrations of the desired reagent. Usually about six different concentrations were employed, and they were kept in tightly stoppered vials. Then, as soon as possible, the cytoplasmic viscosity of the eggs was tested. As my studies progressed, I was able to predict the fate of the various groups of eggs. A certain degree of antigelatinizing action always prevented the formation of the spindle. The weaker solutions, unable to produce the requisite effect on cytoplasmic viscosity, did not prevent the division of the cell. On the other hand, too concentrated solutions produced intense gelation and killed the eggs. Intermediate concentrations gave the desired results. In these cytoplasmic gelation was inhibited or reversed, and viscosity determinations showed the cytoplasm to have a liquid consistency. In such eggs with fluid cytoplasm, no mitotic figure ever formed. Nevertheless, the eggs were not all killed by the reagent, as some were able to resume their development on return to sea-water.

The results obtained with the fourteen substances enumerated above were all similar. Individual differences occurred, but in every case the general scheme was the same. It will scarcely be necessary, therefore, to record all of the experiments, and I shall content myself with presenting only some representative

ones. Moreover, in the tabulation of these experiments, I shall attempt to reduce the data to the briefest possible form, omitting many of the less essential observations.

In order to compare the cytoplasmic viscosity of the treated eggs with that of the normal controls, normal and treated eggs were centrifuged simultaneously in separate tubes of the centrifuge. These comparison tests had to be made before the control eggs segmented, and preferably at a time when the cytoplasmic viscosity of the eggs was high. Thus only a limited period was available, and it was impossible to test the viscosity of the eggs in all the vials. Oftentimes it was necessary to repeat experiments. It was always found that when a particular concentration of a reagent completely prohibited gelation, the eggs subjected to this concentration never formed a spindle. This relation always held true.

In the following description, frequent reference is made to per cent solutions. In most cases, solutions of liquids in liquids are referred to, and in such instances, per cents by volume are understood. By this I mean cc. of solute per 100 cc. of solution. In the few cases when solid substances were dissolved, the per cent indicated is a weight per cent. All solutions were of course made up in sea-water.

Wilson ('01) found that 2.5 per cent ether prevented the appearance of astral rays or spindles. I began my experiments by determining the effect of such a concentration of ether on the fertilized egg.

June 22nd. Some eggs were fertilized at 9:42 A.M. and put into 2.5 per cent ether at 9:52 A.M. At 10:01 A.M. normal and etherized eggs were centrifuged simultaneously, the high-speed handle being turned 30 times in 28 seconds. The normal eggs remained unstratified, the etherized eggs showed a very evident stratification. Thus $2\frac{1}{2}$ per cent ether has an antigelatinizing action. This concentration of ether prevents spindle formation, although it does not otherwise harm the cell. Higher concentrations of ether are injurious. Thus $4\frac{1}{2}$ per cent and 5 per cent ether produce a rapid coagulation, and $3\frac{1}{2}$ per cent and 4 per cent ether, although they at first tend to liquefy the cytoplasm, after an hour or so coagulate it.

Chloroform has a similar effect, as is borne out by the following experiment:

July 10th. Eggs fertilized at 10:16 A.M. were placed in a 0.13 per cent solution of chloroform at 10:26½ A.M. At 10:33 A.M. normal fertilized eggs and some of those in the chloroform solution were centrifuged simultaneously, the handle being turned 50 times in 29 seconds. The normal eggs had a hyaline zone barely indicated, whereas the chloroformed eggs showed a hyaline zone extending halfway along the axis of stratification. The eggs treated with chloroform did not segment.

Various experiments with paraldehyde showed that a 4 per cent solution was very effective in reversing gelation.

July 10th. Eggs fertilized at 11:51 A.M. were transferred to 4 per cent paraldehyde at 12:02 P.M. At 12:09 P.M. normal eggs and those exposed to paraldehyde were centrifuged simultaneously. In the normal eggs the hyaline zone was barely indicated, in the treated eggs it extended through half of the egg. The concentration of paraldehyde used was sufficient to prevent segmentation.

The following experiments deal with the effects of various other substances which act like ether and chloroform.

July 17th. Chloral hydrate. At 12:05½ to 12:06 P.M., eggs fertilized 24 minutes previously (at 11:42 A.M.) were subjected to seven different concentrations of chloral hydrate, varying from $\frac{1}{60}$ per cent to 1 per cent. Of these concentrations, 1 per cent of the reagent produced coagulation, but concentrations of $\frac{1}{4}$ per cent to $\frac{1}{12}$ per cent had the opposite effect and produced reversal of the normal gelation. These concentrations prevent segmentation, but the inhibition is only temporary, and the eggs segment (although often somewhat irregularly) upon return to sea-water.

In the above description many of the less important details of of the experiment were omitted. The following experiment is reported more fully:

July 22nd. Amyl alcohol. At 11:44 A.M., eggs fertilized five minutes previously were put into vials containing various concentrations of isoamyl alcohol (isobutyl carbinol). Six vials were used. *A* contained 2 per cent of the alcohol, *B* 1.33 per cent, *C* 1 per cent, *D* 0.67 per cent, *E* 0.33 per cent, and *F* 0.17 per cent.

At 11:51 A.M., eggs in *A* and normal eggs were centrifuged simultaneously, the handle of the centrifuge being turned 50 times in 29 seconds. The *A* eggs were evidently coagulated, for they showed no stratification; the normal eggs had the hyaline zone barely indicated. At 12:02½ P.M., eggs in *B* and normal eggs were centrifuged simultaneously at the same speed as in the previous test. They, too, gave no evidence of stratification. At 12:12½ P.M., the eggs in *D* and normal eggs were

centrifuged, the handle being turned 50 times in 30 seconds. The *D* eggs had a hyaline zone extending one-third or more of the distance along the stratification axis. In these eggs the gray cap was apparently lacking. In the control normal eggs the original gelation was beginning its normal reversal, and the hyaline zone extended about one-fourth of the stratification axis. At 12:23½ P.M. eggs in *C* and normal eggs were centrifuged simultaneously, the handle being turned 50 times in 30 seconds. Of the *C* eggs almost all were cytolyzed and showed no stratification. A few, however, showed a hyaline zone one-half to two-thirds of the distance along the axis of stratification. Such a marked liquefaction is often the preliminary of coagulation. In the normal eggs at this time, segmentation was beginning, and the hyaline zone was poorly shown.

The eggs in *A*, *B*, *C*, *D*, did not segment. Those in *E* had numerous small cells cut off from the margin. This is an appearance typically found in all cases where the concentration of the anesthetic is not quite sufficient to stop segmentation completely. In *F*, the cleavage was much more nearly normal. These observations were made at about 1:30 P.M. At 12:36 to 12:37 P.M. some eggs from each of the dishes *A* to *F* had been transferred to Stender dishes containing fresh sea-water. Those from *A* were put into *a*, those from *B* into *b*, etc. These dishes were then examined at 1:45 P.M. No segmentation occurred in *a*, *b*, *c*, and in *d* only abnormal evidences of the segmentation process were found. But in *e* and *f* normal segmentation occurred, and motile blastulae were later found in these dishes.

July 24th. Acetonitrile (methyl cyanide). At 11:35½ A.M. eggs were fertilized. Ten minutes later they were placed in vials containing solutions of acetonitrile in sea-water. Vial *A* contained 5 per cent, *B* 4 per cent, *C* 2½ per cent, *D* 2 per cent, *E* 1 per cent, *F* ½ per cent, *G* ¼ per cent. At 11:52 A.M., eggs in *B* and normal eggs were centrifuged simultaneously the handle being turned 50 times in 28 seconds. The *B* eggs had a hyaline zone one-third to one-half the extent of the egg axis, whereas the normal controls showed barely a trace of hyaline zone in a few cases. At 11:59 A.M., eggs in *A* and normal eggs were centrifuged at the same speed as in the previous test. A few of the *A* eggs showed a hyaline zone extending along one-third of the egg axis, but in most cases the eggs were coagulated and showed no stratification. The control of normal eggs exhibited no stratification, except in a few cases which showed trace of a hyaline zone.

At 12:05 to 12:07½ P.M. some eggs were transferred out of *A*, *B*, *C*, *D*, *E*, *F*, *G*, into normal sea-water in Stender dishes *a*, *b*, *c*, *d*, *e*, *f*, *g*, respectively. At 2:15 to 2:30 P.M. eggs in *A* to *G* and *a* to *g* were examined. In *A* and *B*, no segmentation of any kind had occurred. In *C* and *D*, there was no normal segmentation, but in many of the eggs small cell-like masses had apparently been cut off from the cell periphery. In *E*, 10 per cent of the eggs had segmented in more or less normal fashion, others had segmented abnormally. In *F* and *G*, normal segmentation had occurred generally. In *a*, 20 per cent of the

eggs had segmented, some of these had stopped at the two-cell stage, but others had gone on. In *b*, 98 per cent had segmented, and almost all were normal. In *c* to *g* normal segmentation of course occurred.

At about 7 P.M., *a* to *g* were examined for blastulae. In *a*, about 2 per cent of the eggs had developed motile blastulae. In *b* to *g*, practically all the eggs had developed to motile forms, only immature eggs remaining on the bottom of the dish. The eggs in *A* to *G* were then examined. No motile blastulae were found in *A* to *E*, in *F* and *G*, motile blastulae were abundant.

July 28th. Ethyl nitrate. Eggs were fertilized at 11:50 A.M. and at 12 M they were placed in vials *A* to *F*. Vial *A* contained 0.5 per cent ethyl nitrate in sea-water, *B* 0.4 per cent, *C* 0.3 per cent, *D* 0.2 per cent, *E* 0.1 per cent, and *F* 0.05 per cent.

At 12:06½ P.M., eggs in *C* and normal fertilized control eggs were centrifuged simultaneously, the handle being turned 50 times in 30 seconds. The eggs in *C* had a hyaline zone extending through half the egg, in the normal eggs the hyaline zone was not distinct, but was indicated in a fourth of the egg. At 12:19 P.M., eggs in *A* and normal eggs were centrifuged simultaneously, the handle being turned 50 times in 30 seconds. The eggs in *A* showed a hyaline zone extending halfway. In the normal eggs, the cytoplasmic gelation had reversed, and the hyaline zone extended one-third of the distance along the axis of stratification.

At 12:30 P.M., some eggs were removed from each of the vials *A* to *F* and transferred to normal sea-water in Stender dishes *a* to *f*, respectively. In *b* to *f* normal segmentation occurred and motile blastulae were produced. In *a*, some eggs segmented normally, others abnormally; a few motile blastulae resulted. Of the eggs which remained in *A* to *F*, those in *A*, *B*, *C*, did not segment, those in *D*, *E*, *F*, segmented abnormally. No blastulae were produced in *A* to *F*.

It is scarcely necessary to mention other experiments of the same sort which were performed with the various substances previously enumerated. All fourteen substances, in suitable concentration, prevent the appearance of the mitotic figure without otherwise injuring the egg. Those concentrations which act in this way are the very ones which inhibit gelation and preserve the fluid condition of the cytoplasm.

Perhaps the action of these substances depends upon their lipoid-solvent action. This action does not appear to be exerted on the plasma-membrane, as many students of anesthesia have thought, for the vitelline membrane, which I have shown ('15) to be the plasma membrane of the unfertilized egg, is, as far as I can determine, morphologically unaffected. Similarly, the hyaline layer, which becomes the plasma membrane soon after

fertilization, shows no signs of alteration. On the other hand, the lipoids of the egg interior are oftentimes visibly changed. This is easily seen after the egg is centrifuged, for the cytoplasmic lipoids then become aggregated at one pole of the egg, forming there a small polar accumulation known as the gray cap. After the egg had been treated with one of the substances used in the above experiments, it was often noted that the gray cap appeared pale and indistinct. Sometimes the gray cap was apparently absent. Thus these substances which prevent gelation possibly produce their effect by acting on the lipoids of the egg.

Even before Wilson had shown that ether prevented the appearance of asters and spindle, O. Hertwig had made the observation that this effect could be produced by low temperatures (−2° to −3°) without otherwise injuring the egg. My views, therefore, demanded that such low temperatures have a liquefying effect on the cytoplasm. This was fully borne out by experiment.

June 24th. At 4:36 P.M., eggs fertilized sixteen minutes previously were exposed to a temperature of −3°. Fifteen minutes later (at 4:51 P.M.), the eggs were removed from the cold, and after an interval of two minutes they were centrifuged simultaneously with control eggs, also fertilized at 4:36 P.M., but not exposed to cold. The handle was turned 40 times in 30 seconds. On examination, the untreated control eggs showed no stratification whatever, whereas the eggs exposed to cold showed the various stratification zones plainly.

Not only does cold exert an antigelatinizing action on fertilized eggs, but it has a similar liquefying effect upon the cytoplasm of unfertilized eggs.

June 24th. Some unfertilized eggs were exposed to a temperature of −3°, and after ten minutes they were centrifuged simultaneously with normal eggs, the centrifuge handle being turned 21 times in 15 seconds. Both sets of eggs showed stratification. In the normal eggs, however, the granular zone was not distinct from the pigment zone, whereas in the cold-treated eggs the pigment granules had migrated more completely, thus effecting a separation between granular and pigment zones.

The question now arises as to how this antigelatinizing effect of cold is produced. Obviously it cannot act act as a lipoid solvent. The idea suggested itself, however, that cold might pro-

duce the same effect that fat solvents do, but in quite a different way. Possibly low temperatures tend to precipitate the fat globules out of the cytoplasmic emulsion. Such a precipitation might produce an effect comparable to that of the lipoid solvents, for either precipitation or solution would tend to remove the lipoids from their emulsified state. If cold and lipoid solvents both produce their effect by acting on the lipoids of the cell, it is evident that these effects, instead of being complementary, would be antagonistic. Actual experiment demonstrated an antagonism between cold and ether. Eggs treated both with cold and ether showed less antigelatinizing effect than when treated with cold alone or with ether alone.

EFFECT OF INCREASED GELATION

The preceding discussion was concerned with the effects of various antigelatinizing agents upon the cytoplasm. It might be interesting to mention briefly some other experiments with substances which tend to intensify the normal gelation. Many authors have investigated the effect of hypertonic solutions on dividing eggs. Loeb ('92) and Norman ('96) found that if the hypertonic solution was sufficiently strong, cleavage was stopped. Oftentimes, nuclear division without cytoplasmic division resulted.

The effect of hypertonic solutions was investigated by the centrifuge method, and in all cases an intensified gelation could be demonstrated. This is apparently especially marked in the cortex of the egg, and it is probable that such a cortical gelation is the main factor which inhibits division of the cell, even when the nucleus is still able to divide mitotically.

July 5th. Eggs were fertilized at 10:43½ A.M. At 11:08 A.M. some of these eggs were placed in Stender dishes *A*, *B*, *C*, *D*, *E*.

A contained 40 cc. sea-water plus 2 cc. 2½ N NaCl
B contained 40 cc. sea-water plus 4 cc. 2½ N NaCl
C contained 40 cc. sea-water plus 6 cc. 2½ N NaCl
D contained 40 cc. sea-water plus 8 cc. 2½ N NaCl
E contained 40 cc. sea-water plus 10 cc. 2½ N NaCl

At 11:17 A.M., eggs from *C* were centrifuged simultaneously with a control lot of untreated fertilized eggs, the handle being turned 50 times in 29 seconds. Whereas the normal eggs had the hyaline zone well indicated, the eggs from *C* showed not a trace of stratification.

At 11:25½ A.M., eggs from *B* were compared with normal control eggs. The centrifuge handle was turned 50 times in 29 seconds. The normal eggs showed a hyaline zone extending a third of the distance along the axis of stratification. The *B* eggs showed not a trace of a hyaline zone.

At 11:36½ A.M., eggs from *A* were compared with normal eggs. The centrifuge handle was turned 50 times in 28 seconds. The normal eggs showed a prominent hyaline zone, extending at least a third of the distance along the axis of stratification. In the *A* eggs, the hyaline zone was barely indicated.

At 11:53 A.M., the eggs from *D* and from *E* were centrifuged, the handle being turned 50 times in 30 seconds. Neither eggs from *D* nor those from *E* showed any trace of stratification.

Thus it is evident that the addition of hypertonic NaCl to sea-water, has the effect of intensifying the gelation of the egg cytoplasm.

At 12 M, the control of untreated fertilized eggs contained eggs in the two-celled stage, but there was no segmentation in *A*, *B*, *C*, *D*, *E*. At 2 P.M., these dishes were again examined. At this time cleavage was occurring in *A*. In *B* there was nuclear division without cytoplasmic division. In *C*, *D*, and *E* there was neither nuclear nor cytoplasmic division.

At 2:24 P.M., eggs from *B* and from *C* were centrifuged, the handle being turned 50 times in 28 seconds. In the *C* eggs the hyaline zone was indicated in some eggs, but not very clearly. In the *B* eggs the hyaline zone was prominent, extending through about one-third of the egg. But it was not very transparent, for a cortical zone of granules covered it. This indicates a cortical gelation. It will be remembered that the *B* eggs are those in which nuclear division without cytoplasmic division was found to take place.

The effect of potassium cyanide is worth recording. Even in very dilute concentrations, the cyanide inhibits cell division. But, curiously enough, the early phases of mitosis are able to continue in such concentrations. Likewise, the final stages of the process can go on. The explanation that I would offer is a very simple one. Potassium cyanide intensifies gelation and the normal gelation is rendered irreversible. This can be shown true by viscosity measurements.[1]

[1] Moreover, it is directly in line with my previous observation that cyanide prevents swelling of the gel which forms the vitelline membrane of the egg.

In order to determine the concentration of KCN necessary to suppress division, I made a 0.004 per cent solution[2] in sea-water by diluting a 1 per cent solution (in distilled water) with 249 parts of sea-water. This 0.004 per cent solution was successively diluted with equal parts of sea-water until nine solutions were obtained, each half the concentration of the preceding one. Eggs were subjected to all these solutions, and it was found that a concentration of 0.000625 per cent sufficed to check segmentation. In concentrations of 0.0000313 per cent and 0.0000156 per cent, segmentation proceeded to about the four-cell stage, but then went no further.

The following experiment shows that the early stages of mitosis can occur in concentrations much above those which inhibit the entire process:

June 25th. Eggs were fertilized at 5:18½ P.M. In these eggs the spindle first began to be visible twenty minutes later (at 5:39 to 5:40 P.M.). At 5:23 P.M., 5:27 P.M., 5:32 P.M., 5:38 P.M., some of the fertilized eggs were transferred into Stender dishes *A*, *B*, *C*, *D*, respectively. Each of these dishes contained 0.0025 per cent KCN, prepared by diluting 1 per cent KCN (in distilled water) with 399 parts of sea-water.

The eggs in *A* were observed at 5:47 P.M. Instead of showing a small nucleus, they showed a large pale spot with a vague border. This spot was often elongated, and probably represented an abnormal spindle. When the eggs in *B* were examined at 5:58 P.M., they showed a pre-spindle plainly, and they appeared much like the normal eggs. None of the eggs in *A*, *B*, *C*, *D*, proceeded to develop any further than the spindle stage, and observation at 10:40 P.M. showed them all with spindles, but unsegmented.

It might be thought that the above experiment owes its explanation to the fact that the cyanide penetrates the eggs slowly, and that only after a time is its influence felt. However the eggs in *D* appeared to be checked almost immediately. Moreover, a later experiment showed that this interpretation could not be the correct one. In this case the fertilized eggs were put into a 0.004 per cent solution of KCN in sea-water five minutes after fertilization. The eggs in the KCN solution were kept in a test-

[2] Of course the actual concentration of KCN is not referred to, as there is a reaction between KCN and the salts of sea-water.

tube tightly sealed with a rubber stopper, and the test-tube was then exposed to a temperature which varied from 10° to 12°. No spindle appeared while the eggs were in the cold, but when the test-tube was removed from the cold after an exposure of two hours and thirty-eight minutes and warmed with the hand, then spindles soon made their appearance. Thirteen minutes later they could be observed plainly. During their two and one-half hours' stay in the cold, the eggs must have been thoroughly penetrated by the KCN solution, and yet as soon as they were placed in a warmer temperature, development proceeded as far as the spindle stage. Of course no segmentation occurred in these eggs.

The following experiment shows that in a 0.005 per cent solution of KCN, the final stages of mitosis can proceed:

August 23rd. Some eggs were fertilized at 8:23 P.M. and they first began to segment forty-two minutes later. At 30, 33, 35, 38, 40, 42 minutes after fertilization these eggs were removed to stoppered test-tubes *A*, *B*, *C*, *D*, *E*, *F*, respectively, each of which contained 0.004 per cent KCN in sea-water. Counts of the segmenting eggs showed in *F*, $\frac{96}{100}$; in *E*, $\frac{94}{100}$; in *D*, $\frac{88}{100}$; in *C*, $\frac{96}{100}$; in *B*, $\frac{94}{100}$; in *A*, $\frac{59}{100}$; normal control eggs, $\frac{94}{100}$. In these fractions the numerator represents the number of segmenting eggs counted, the denominator, the total number of eggs observed. Thus it is apparent that for the last twelve minutes the cleavage process is able to continue in the presence of 0.004 per cent KCN.

In order to explain this curious action of KCN on one particular stage of mitosis, I have already suggested that the cyanide renders irreversible the normal gelation. Even before the above experiments were performed, I had evidence supporting this view.

June 25th. Some eggs fertilized at 2:54 P.M. were at 3:14 P.M. subjected to the action of 0.005 per cent and 0.0025 per cent KCN. At 3:34 P.M. eggs in 0.005 per cent KCN were centrifuged simultaneously with normal eggs, the handle of the centrifuge being turned 45 times in 30 seconds. The eggs exposed to the cyanide showed no stratification whatsoever, whereas the normal eggs, as expected, showed a very distinct stratification. A later test of the eggs in 0.0025 per cent KCN gave similar results. Thus the KCN prevents the normal reversal of gelation.

The results with KCN lend additional support to the views already expressed on the relation of the mitotic process to the colloidal changes in the cytoplasm. Cyanide acts by intensifying gelation. Hence, as is to be expected, it does not (in moderate concentration) prevent the early stages of mitosis, and development proceeds as far as the spindle stage. But that particular stage of mitosis which is associated with a reversal of gelation cannot take place in the presence of the cyanide.

Chloretone acts somewhat like KCN. A 0.08 per cent solution checks segmentation, although it does not markedly injure the eggs. Such a solution intensifies the normal gelation.

THE NATURE OF THE NORMAL MITOTIC GELATION

Earlier in this paper I have attempted to show that the appearance of the mitotic figure is necessarily preceded by a cytoplasmic gelation. Such a gelation can be artificially produced in unfertilized eggs by various reagents, the best of which apparently is a hypertonic solution. The question now arises as to how the gelation occurs normally. The fact that its artificial production appears to be best imitated by hypertonic solutions leads to the suggestion that, similarly in the developing egg, gelation and consequent formation of astral rays and spindle is initiated by the abstraction of water from the cytoplasm by the growing pronuclei. Some of the early students of artificial parthenogenesis thought that the essential step in the initiation of development was the abstraction of water from the cytoplasm.

If the normal gelation is produced by an excessive salt concentration, then it should be possible to show that gelation produced artificially by hypertonic solutions behaves like the normal gelation. To a certain extent this has been done. When the cytoplasm of the unfertilized egg is gelatinized by hypertonic solutions, such a gelation can be reversed by ether. On the other hand, ether has no effect in reversing or antagonizing the gelatinizing (or coagulating effect) of acids or of distilled water. Hence of these three types of gelation, that produced by hypertonic solutions behaves most nearly like the normal.

June 28th. At 9:38 A.M., 8 cc. of $2\frac{1}{2}$ N NaCl were added to 50 cc. of sea-water containing unfertilized eggs. At 10:39 A.M., some of these eggs were removed to a solution containing 3 per cent ether. This solution was made up by adding to $43\frac{1}{2}$ cc. of sea-water, 5 cc. of $2\frac{1}{2}$ N NaCl, and $1\frac{1}{2}$ cc. of ether.

At 10:$49\frac{1}{2}$, the cytoplasmic viscosity of the eggs in both solutions was compared by a simultaneous centrifuge test, by which it was found that the eggs in the solution containing ether had a more fluid cytoplasm. The centrifuge was turned 45 times in 28 seconds. After this treatment, the eggs in the hypertonic solution without ether showed no sign of stratification, whereas the eggs which had for ten minutes been exposed to ether (although still in a hypertonic solution) showed the beginnings of stratification. In them, the pigment granules had shifted somewhat, the gray cap and hyaline zone were beginning to appear.

That ether exerts an antagonistic action toward hypertonic solutions was shown in still another way. As has been previously pointed out, $2\frac{1}{2}$ per cent ether reverses the normal gelation and thus prevents the appearance of the mitotic figure. However, when $2\frac{1}{2}$ M NaCl was added, although the concentration of ether remained the same, the ether was no longer able to repress the formation of spindles and asters.

In several experiments I tried to discover if ether would prevent the gelation of the egg cytoplasm by distilled water or by acid. Rather than a decrease, the addition of ether apparently produced a slight increase in the gelatinizing power of dilute acid solutions. The following experiment serves as a sample:

July 20th. At 5:20 P.M., unfertilized eggs were placed into Stender dish *A*, which contained 50 cc. of sea-water plus 1.3 cc. $\frac{N}{10}$ HCl, and also into Stender dish *B*, which contained 50 cc. of 2 per cent ether dissolved in sea-water + 1.3 cc. $\frac{N}{10}$ HCl. At 5:25 P.M. the cytoplasmic viscosity of the eggs in *A* and *B* was simultaneously tested with the centrifuge, the handle being turned 35 times in 30 seconds. Many of the eggs from *A* were injured. The intact eggs showed a hyaline zone extending one-fourth of the distance along the axis of stratification. The eggs from *B* were coagulated and showed not a trace of stratification. At 5:35 P.M., eggs from *A* and *B* were again centrifuged, the handle being turned 35 times in 28 seconds. This time both sets of eggs were coagulated, and in neither case was any stratification visible.

The following experiment, although perhaps not conclusive, indicates that ether does not prevent gelation of egg cytoplasm by distilled water:

June 29th. At 11:38 A.M., some unfertilized eggs were dropped into distilled water, and one minute later they were transferred out of the distilled water into *A*, which contained pure sea-water, and *B*, which contained 2½ per cent ether in sea-water.

At 11:50, the eggs in *A* and *B* were centrifuged simultaneously, the handle being turned 50 times in 30 seconds. When the centrifuged eggs from *A* and *B* were compared, both lots appeared the same. In both cases most of the eggs showed stratification, with a wide and distinct hyaline zone. In both cases, a considerable number of the eggs were cytolyzed and showed not a trace of stratification.

If the normal gelation is due to an abstraction of water, then it should be possible to show an antagonism between cold and hypertonic solutions, whch would be comparable to the effect of cold on the normal gelation. So far, my results in this direction have not been completely successful. Although I have been able to show that cold retards the gelatinizing effect of hypertonic solutions on the unfertilized egg, I have not yet demonstrated that cold can cause a reversal of gelation when once this has been produced by hypertonic solutions. But only a single experiment has been tried, and perhaps further observation will also show this to be true. The following experiments shows that cold tends to prevent gelation of the cytoplasm by a hypertonic salt solution:

August 30th. A hypertonic solution was prepared by adding 8 cc. of 2½ n NaCl to 50 cc. of sea-water. This solution was then divided into two portions, of which *A* remained at room temperature, and *B* was kept at a temperature which varied from $-1.3°$ to $+1°$. At 10:58 A.M., unfertilized eggs were placed in *A*. At 11:08 A.M., some of the eggs in *A* were removed to *B*. At 12:31 P.M., eggs in *A* and *B* were centrifuged simultaneously, the handle being turned 50 times in 30 seconds. The eggs in *B* showed a gray cap and a hyaline zone extending about one-third of the distance along the axis of stratification. The eggs in *A* showed not a trace of stratification.

If the gelation which occurs normally is due to water abstraction, it might be also expected that when the egg is made to take up water, this gelation could be reversed. This is in fact true, and hypotonic solutions effectually cause a reversal of gelation in the fertilized egg. This was shown clearly by centrifuge tests. Because of this antigelatinizing effect, hypotonic solutions act like ether and prevent segmentation without otherwise injuring the egg.

June 28th. Eggs fertilized at 11:31 A.M., were at 11:46½ A.M., dropped into Stender dish *B*, which contained 40 cc. sea-water plus 10 cc. distilled water. The untreated fertilized eggs remained in Stender dish *A*. At 11:51½ A.M., eggs in *A* and *B* were centrifuged simultaneously, the handle being turned 45 times in 26 seconds. The eggs in *A* showed just the beginnings of a hyaline zone. The eggs in *B* were markedly more stratified, they showed gray cap and hyaline zone plainly. In this experiment the sea-water was not sufficiently dilute to prevent segmentation. In another experiment it was found that a solution made up of equal parts of sea-water and distilled water was the most favorable for the reversible prevention of cleavage. In such a solution, eggs remained unsegmented, and yet after a three-hour exposure, they were able to resume their development when returned to normal sea-water.

SUMMARY

1. During the period between fertilization and the first cleavage of the sea-urchin egg, the viscosity of the cytoplasm rises until it reaches a maximum, then it decreases again.

2. Similar viscosity changes occur in relation to the second cleavage.

3. The changes in viscosity are very marked and indicate the occurrence of a gelation in the cytoplasm.

4. This gel-formation reaches its height just before the spindle appears. Later the cytoplasm becomes more fluid again.

5. That gelation is not secondary, but is a predetermining factor in spindle or aster formation, is indicated by the fact that when gelation is suppressed, the mitotic figure does not form, although the eggs may be otherwise uninjured.

6. Such suppression of gel formation was produced by fourteen different substances, all lipoid solvents.

7. It can also be produced by cold.

8. Although they produce the same effect, the action of cold and of lipoid solvents is mutually antagonistic.

9. The effect of hypertonic solutions on dividing eggs can be interpreted on the basis of the fact that they increase the cytoplasmic viscosity. Potassium cyanide and chloretone also act in this way.

10. The cytoplasmic gelation which occurs in relation to mitosis is apparently due to an abstraction of water, for—

a. It can be most closely imitated by an abstraction of water. Cytoplasmic gels produced by hypertonic solutions on unfertilized eggs behave toward cold and ether much like the normal gel of the fertilized egg. Cytoplasmic gels produced by acid or by distilled water do not exhibit this resemblance.

b. Entrance of water into the fertilized egg reverses the normal cytoplasmic gelation.

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Resumen por el autor, Caswell Grave.

Amaroucium pellucidum (Leidy), forma constellatum (Verrill).
I. Movimientos y reacciones de la larva.

El cuerpo de la larva, que como es sabido se asemeja por su forma a los renacuajos de los anfibios, presenta un movimiento de rotación en el sentido de las agujas de un reloj, alrededor de su eje, durante la locomoción, a consecuencia de la forma asimétrica del cuerpo o de la torsión del eje de la cola. Las larvas presentan una reacción positiva definida hacia la luz durante un intervalo muy breve que sigue inmediatamente a su liberación de la colonia que las produjo, pero reaccionan negativamente hacia la luz durante la mayor parte del periodo de actividad ulterior. Las larvas permanecen invariablemente en la superficie del agua o cerca de ella durante la primera parte del periodo de actividad, pero al aproximarse el tiempo de la metamorfosis descienden a las capas inferiores y nadan en el fondo o cerca de él. La reacción positiva de la gravedad se presenta con varios grados de intensidad, faltando completamente en un pequeño tanto por ciento de las larvas. Hacia el final del periodo de actividad, el contenido viscoso de los extremos glandulares de las papilas adhesivas se vierte sobre la superficie externa de la túnica, y la fijación inicial de la larva acaece cuando una o varias de estas gotitas viscosas se ponen en contacto con la superficie de un cuerpo extraño. La duración del periodo de natación libre de la larva varía desde diez minutos hasta dos horas.

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AMAROUCIUM PELLUCIDUM (LEIDY) FORM CONSTELLATUM (VERRILL)

I. THE ACTIVITIES AND REACTIONS OF THE TADPOLE LARVA

CASWELL GRAVE

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FOUR FIGURES

A study of the organization, activities, and metamorphosis of the free-swimming larva of Amaroucium pellucidum constellatum was begun during the summer of 1912 at the Marine Biological Laboratory at Woods Hole, Massachusetts, and has been continued as opportunities have permitted. It has also included work on the structure and asexual reproduction of the primary ascidiozooid and the formation of colonies and the differentiation of germ cells by the secondary ascidiozooids.

The observations made on the activities and reactions of the tadpole during its free-swimming period are recorded in this paper. The histological structure of the organs involved in larval activities will be described and figured in a paper now being prepared for publication on the organization of the Amaroucium tadpole.

LOCOMOTION

Since the discovery of the chordate affinities of ascidians by Kowalevsky, in 1866, a closer similarity in the behavior, as well as the fundamental structure, of the ascidian tadpole with chordate animals has been taken for granted than is apparently warranted. In the numerous papers which have been published on the development and metamorphosis of ascidians very few observations on the behavior of the free-swimming larva have been recorded. MacBride,[1] in his Text Book of Embryology, page 619, states that the tadpole larva propels itself like a fish

by lateral blows of its tail. In the course of my study of the Amaroucium tadpole, it was a great surprise to find that this larva does not swim in the fashion of a vertebrate, in which a constant position of the body is maintained, but that the body of the tadpole while swimming is in constant and rapid rotation on its long axis, clockwise as seen from behind. I have found this method of locomotion also in the tadpole larva of Botryllus and suspect it is characteristic of the larvae of ascidians in general. On account of the suddenness with which the tadpole begins to swim after one of its quiescent intervals, and the rapidity with which it passes through and out of the field of vision with the microscope, the rotary movement of the body during locomotion is difficult to observe; however, by the addition of a narcotizing agent to the water, such as a solution of Epsom salts, the rapidity of the swimming movements of the tadpole is gradually reduced and, when moving slowly, the revolutions of the body are readily noted.

The mechanism by which the body is caused to rotate is also not immediately evident. The body of the tapole is somewhat compressed and, when at rest, comes to lie on one of its flattened sides. For convenience in description, these flattened surfaces will be referred to as the right and left sides. The side of the body containing the rudiments of the oral and atrial siphons and the pigmented sense organs will be referred to as dorsal, the tail as posterior in position, and the end opposite to tail, containing the adhesive papillae, as anterior.

It is generally assumed that the tail fin of the ascidian tadpole is expanded vertically, as in vertebrates, and in general this may be the case, but the tail fin of the Amaroucium tadpole is *horizontal* in position.

Seeliger[2] noted that the tail of the larva of Clavelina lepadiformis, in consequence of its forward growth along the side of the body beneath the closely fitting chorionic membrane, becomes twisted and that the nerve tube is thus turned from the dorsal to the left side. According to his descriptions and figures of the free-swimming tadpole, however, it follows that the tail untwists when the chorion is burst, the nerve tube assuming the

dorsal, the tail fin the vertical position. Seeliger states also that this larva swims in the manner of (amphibian) tadpoles.

The slight asymmetry which exists between the right and left sides of the body may account for the rotation of the body as it is propelled through the water by the lashing of the tail. Viewed from the dorsal side, a concave depression is seen on the left located near the anterior end; moreover, the anterior tip of the body, containing the middle adhesive papilla, is found to lie slightly to the right of the median sagittal plane (fig. 1). These asymmetrical features are the result of the pressure of the tail which, during the embryonic period of development, is folded forward beneath the chorionic membrane and coiled about the anterior part of the body. The depression takes an oblique course from below upward across the left side of the body and therefore gives to it the form of a screw with a single groove which tends to set up an axial rotation in the observed left to right direction when the body is propelled rapidly through the water.

Considerable support for the suggestion that the screw-like form of the body plays a part at least in producing rotation during locomotion is found in the fact that when a tadpole suddenly stops swimming, as it frequently does during its active period, the axis of the body quickly assumes a vertical position, tail upward, and begins to revolve slowly from left to right as it slowly sinks through the water.

The lateral asymmetry of the body may not be the only or even the chief factor in producing rotation during locomotion. The direction and character of the strokes of the tail may be such as to cause rotation, and my observations on the character of the movements of tadpoles held captive beneath a cover-slip, and on tadpoles in which a part of the tail has been amputated, lead me to believe the strokes of the tail are not made directly to the right or left, but that the contractions of the muscle fibrillae, due to their orientation in the muscle cells, tend to slightly twist as well as to bend the axis of the tail, thus producing a spiral thrust. The disposition of the contractile fibrillae in the cortical layer of the muscle cells is shown in figure 2. As

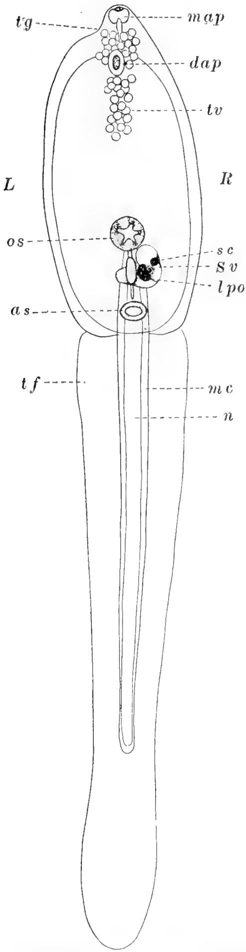

Fig. 1 Camera outline drawing of the fully developed tadpole larva of Amaroucium pellucidum constellatum as seen from the dorsal surface, showing the lateral asymmetry of the body, the horizontal position of the tail fin, and the location of the sense organs in the sensory vesicle.

was noted and figured by Kowalevsky[3] and Seeliger, the muscle fibrillae take a slightly oblique course in each cell and the fibrillae of adjacent muscle cells seem to be continuous from cell to cell.

When a tadpole is lightly held between a slide and cover-slip, the vibrations of the tail cause the body to oscillate, the anterior end seeming to move in a circular or oval path on a pivot located near the posterior part of the body. When a part of the tail is amputated, the vibrations of the remaining stump do not pro-

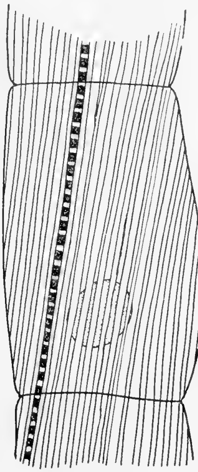

Fig. 2 Camera outline drawing of a muscle cell, showing the oblique position of the fibrillae in the cortical layer, the continuation of the fibrillae from cell to cell, and the cross striations in one fibrilla.

ABBREVIATIONS (FIG. 1)

as, atrial siphon
dap, dorsal adhesive papilla
L, left side
lpo, light-perceiving organ
map, middle adhesive papilla
mc, muscle-cell sheath
n, notocord
os, oral siphon
R, right side
sc, statolith cell
sv, sensory vesicle
tf, tail fin
tg, groove in test resulting from pressure of the tail during embryonic development
tv, test vesicles

duce locomotion, but throw the body into vibration with the appearance of oscillatory movement the same as that seen when locomotion in a normal tadpole is prevented by confining it between slide and cover.

The rotary method of locomotion in the ascidian tadpole may be accidental and have no special significance, but it is of some interest to find its method of swimming is that characteristic of the early larvae of invertebrates in general and not like that of vertebrates.

REACTIONS TO LIGHT

When colonies of Amaroucium are placed in a rectangular glass jar lined except for one side with unglazed black paper and the jar is so placed that light enters the water through the uncovered side, the tadpole larvae, as they are liberated, swim through the water first in an undulatory course obliquely upward toward the light, then up along the side of the glass to the surface of the water where they dodge back and forth for a few seconds attempting to proceed toward the source of the light. They then leave the illuminated side and swim into the less illuminated parts of the jar, where they remain, alternately active and quiescent, throughout their free-swimming period.

Castle[4] noted that "Ciona and Amaroucium tadpoles avoid the light while that of Botryllus swims toward light." He failed to observe the Amaroucium tadpole during the brief interval when, on escaping from the parent colony, it shows a positive light reaction.

Occasionally a tadpole, in its initial excursion toward the light, will turn in its course and swim away before it has reached the lighted side of the jar. That is, the period of positive reaction to light in some tadpoles is remarkably short, but is apparently never entirely absent.

Tadpoles do not swim in a straight course either toward the source of light, during the brief period when they react positively to it, or away from its source during their longer period of negative response, but depart from a straight line in all directions and, at times, they travel in circles and curves of con-

siderable magnitude, as is shown by the diagram (fig. 3). When observing the movements of a single tadpole, especially during the period of its negative reaction to light, it is difficult to follow the exact path and trend of its course, but, when working in a dark-room with a large number of tadpoles in a rectangular dish with electric light bulbs at opposite ends of the dish, the nega-

Fig. 3 Showing three characteristic paths taken by tadpoles upon emerging from the parent colony during exposure in a rectangular jar 12 cm. in width, 18 cm. in length, and 25 cm. in depth, to bright directive light reflected from a south window, 12 feet distant, as seen from above. The sides of the jar, *mo*, *op*, and *pn*, lined on the inside with black unglazed paper. *I* and *II*, Amaroucium colonies on the bottom of the jar. *a*, *b*, and *c*, points at which tadpoles emerged. Dotted lines, paths taken by tadpoles. Arrows indicate width and direction of beam of light.

tive response of the group as a whole is strikingly definite and immediate. The entire group moves slowly away from the source of light, changing its direction the instant the source of the light is reversed.

The light-perceiving organ of the tadpole, composed of a series of three lenses, a cup-shaped layer of pigment granules, and a

group of retinula cells, is not located in the median sagittal plane of the body, but is displaced to the right and so oriented that those rays of light only which enter the series of lenses from the upper right side of the body will reach the pigment cup and be effective in stimulating the sensitive ends of the retinula cells (fig. 1). In its natural habitat, light rays of greatest intensity reach the tadpole from the direction of the water surface and, as a consequence of the axial rotation of the body during locomotion, the tadpole receives its maximum stimulations from light at one instant only in each revolution when the body is so oriented that its long axis is parallel to the water surface. It is thus obvious that a series of frequent orienting responses is provided for.

RESPONSE TO GRAVITY

During the first part of their free-swimming period, tadpoles tend to seek and to remain at or near the surface of the water, but later on they swim away from the surface and seem to seek the deeper strata of water and, at the close of the free-swimming period, usually become attached to the less illuminated places on or near the bottom.

The tendency of tadpoles to remain at the surface during the first part of their active period is subject to the interpretation that it is simply one of the results of their positive reaction to light, while their behavior a little later, when they exhibit a very definite negative response to light yet continue to remain at the surface of the water, warrants the conclusion that tadpoles are sensitive to conditions other than light, probably to their position with reference to gravity, but possibly to changes in density or water pressure or to differences in the oxygen or carbon-dioxide content of the upper and lower strata of water.

I incline to the view that the response of the tadpole is to gravity, because of the presence of a statocyst-like structure within the sensory vesicle and the similarity of this organ to sensory structures in other animals known to be end-organs for the perception of the position of the body with reference to gravity.

In order to determine the character and definiteness of the response of the Amaroucium tadpole to gravity, a number of experiments were carried out, in each of which a large number of tadpoles, collected and used as soon as possible after their emergence from the parent colonies, were placed during their free-swimming and attachment periods in a glass cylinder containing a column of water 5.5 cm. in diameter and 40 cm. in depth, note being made of the levels at which the tadpoles became attached.

The results of twelve of these experiments are shown in table 1. The special conditions under which each experiment was made are stated in the explanation of the table.

While these experiments were designed primarily for the study of the response of tadpoles to gravity, those numbered from 1 to 6 in the table show also the effect upon this response of special or unusual conditions of light, and the experiment numbered 12 shows the effect of the absence of light in modifying the normal geotropic response.

Five of the experiments, those numbered from 7 to 11 in the table, were made under conditions considered as nearly normal as it is possible to make them in the laboratory. The unjacketed clear glass cylinder stood 2 feet directly in front of a north window on a table the top of which was $5\frac{1}{2}$ feet below the top of the window and 8 inches below the window sill. The rays of light entered the column of water more or less obliquely from the general direction of the window and were therefore somewhat directive. Combining and summarizing the results of these experiments, it is found that 77.6 per cent of the total 496 tadpoles used in the experiments became attached either to the bottom or to the edge of the bottom on the least illuminated side of the cylinder; 9 per cent to the side below the middle; 7.6 per cent to the side above the middle; 3 per cent to the side of the cylinder at the edge of the water surface, and 2.6 per cent remained floating on the surface of the water, perhaps attached to or held by the surface film.

Notes made during the course of one of the experiments will serve to show the nature of the activities observed:

TABLE 1

Showing the levels at which tadpoles became attached when subjected to various light conditions in a 1000-cc. glass cylinder filled to the 40-cm. mark with sea-water

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*cm.*
SF....	25	14	61[1]	56[1]	32	5	3	1	2	2	5	58	*40*
SE....	12	4	42	21	57	1	1	4	3	3	4	107	
	2	1				2		3				2	*35*
	1	1			1	2		3	1	1		3	*30*
				3		1	2	3				1	*25*
				1		1	3	2	1		1	2	*20*
		1	1	2			3	2		1		1	*15*
				2		1	2	2			1	1	*10*
	1	2		2			4		4	1		1	*5*
		1		5			24	13	5	2	1	3	
BE....	17	30	1	40	10	43	25	52	121	60	81	4	
B.....	13	7	3	19	2	36	8	14	12	5	7	132	
	71	61	108	151	102	92	75	99	149	75	100	315	

[1] The number of tadpoles remaining at the top in experiments 3 and 4 seems abnormally large. It is possible that some of the tadpoles were not in good condition due to the fact that the parent zooids had been kept too long under laboratory conditions.

September 12, 1917. 4.45 P.M. All tadpoles liberated by an Amaroucium colony during a fifteen-minute interval, seventy-five in number, collected in a glass cylinder and placed upright on a table before a north window. All tadpoles swimming actively at the surface of the water. A tadpole dives downward now and then to a depth of 2 to 3 cm., but returns immediately to the surface.

4.53 P.M. One tadpole dived to a depth of 6 cm. and then returned to the surface. Others do the same. None diving deeper.

4.55 P.M. One tadpole swam straight down to a depth of 32 cm., stopped swimming, turned tail upward, and slowly sank to the bottom, where it remained motionless.

4.58 P.M. One tadpole descended to a depth of 9 cm., came in contact with the side of the cylinder and became attached, not able to free itself by repeated attempts to swim away.

5.01 P.M. One tadpole swam half way to the bottom, turned and swam to the top.

5.03 P.M. Several tadpoles dived to depths of about 15 cm., all but one returning to the top, one sank motionless to the bottom.

5.08 P.M. Several tadpoles swimming about actively at and near the bottom, others at the top and in the upper strata of water, one sinking motionless with metamorphic changes taking place.

5.13 P.M. Several tadpoles swimming at various depths between the top and bottom.

EXPLANATIONS, TABLE 1

SF, attached to surface film (floating); *SE*, attached at line in which water surface and side of cylinder meet; *BE*, attached along the bottom edge; *B*, attached to the bottom; figures in the columns indicate the number of tadpoles attached at various levels; figures at the bottom of the columns show the total number of tadpoles used in each experiment.

Experiments 1 and 2 made simultaneously with tadpoles taken from the same colonies of ascidians. This is the case also with experiments 3 and 4 and with 5 and 6.

Experiments 1 and 3 Sides and top of the cylinder covered with black paper. Light from a north window reflected directly upward through the column of water.

Experiments 2 and 4 Sides and bottom of the cylinder covered with black paper. Light from a north window reflected directly downward through the column of water.

Experiment 5 Same as 1 and 3, except that direct rays from the sun were reflected upward through the column of water.

Experiment 6 Same as 2 and 4, except that direct rays from the sun were reflected downward through the water.

Experiments 7 and 8 The cylinder without cover set before a north window.

Experiments 9, 10, and 11 Same as 7 and 8, except the cylinder was inclined at an angle of 45° from the perpendicular.

Experiment 12 Cylinder placed in a dark room during the free-swimming and attachment periods of the tadpoles.

5.30 P.M. A tadpole sinking motionless, tail upward, the body revolving slowly on its long axis, became active and swam to the top.

5.45 P.M. A few tadpoles actively swimming in the upper half of the cylinder, some near the bottom, many have become attached.

6.00 P.M. All tadpoles attached, a few at and near the top, some to the side, but the greater number to the bottom or the edge of the bottom on the side of the cylinder least illuminated.

Tadpoles are alternately active and quiescent during the free-swimming period and, as may be noted from observations just recorded, slowly sink during their quiescent periods, and are thus carried passively from points at various levels into deeper strata of water or even to the bottom. This fact at once suggests the possibility that the proportionately large number of tadpoles which become attached to the bottom may include many that cannot be said to have migrated from the upper surface in consequence of a positive response to gravity. To test this possibility, several experiments were carried out which are similar to those just described in every way except that the cylinder was inclined 45° from the vertical. In a cylinder thus inclined tadpoles which come to the end of their free-swimming period or become quiescent while in the upper strata of water, or at any level higher than 8 cm. above the bottom, can sink a short distance only before reaching and becoming attached to the side. The bottom will be reached by those only which actively seek it out.

The results of these experiments, recorded in columns 9, 10, and 11 of the table, are practically identical with those in which the cylinder stood upright, recorded in columns 7 and 8, showing rather conclusively that tadpoles become attached to surfaces situated considerable distances below the top of the water and to the bottom, not because they passively sink from the higher levels, but in consequence of a definite positive response to gravity.

The cause or causes of the reversal in the geotropic response of tadpoles from negative to positive have not been discovered. The suggestion that a change in the position or orientation of the statolith cell in the wall of the sensory vesicle possibly takes place which may be correlated in some way with the change in

the physiological response to gravity, has been followed up without result. The position of the statolith cell in the wall of the sensory vesicle of tadpoles fixed and preserved late in their free-swimming period is not observably different from that in tadpoles preserved at the time of their liberation from the parent colony. Neither is there any change in the relation of the sensory vesicle as a whole to adjacent structures. Slight contractions in the body wall may be observed during resting periods, but there is apparently no observable shifting of structures within the body until the free-swimming period is over and metamorphic changes have set in.

In the experiments made to demonstrate the existence of a definite reaction to gravity, distinct from that to light, unusual and abnormal light conditions were arranged and the results, recorded in columns 1 to 6 of the table, seem to show that light when reflected directly downward or upward through the column of water in a cylinder, jacketed with black paper to prevent the entrance of light from the side, has a very decided effect in modifying the response of the tadpoles to gravity.

In table 2, which follows, the results of these experiments are given in the form of percentages of tadpoles found attached at various levels in the cylinder.

A comparison of these figures with those given in the text on page 247 will show the extent to which the normal response to gravity is modified in the presence of unusual light conditions.

Not only do tadpoles vary greatly in the degree to which they exhibit a positive response to gravity, about 5 per cent not showing it at all, but the response is such that it may be more or less suppressed by the stronger negative response to light. When light from a north window was reflected upward through the column of water, during the period when 77 per cent of the tadpoles normally migrate to the bottom, the response to gravity was inhibited in more than 58 per cent of the tadpoles, about 19 per cent only finding attachment to the bottom. An even greater inhibition of the response took place when direct sunlight was reflected upward, 11.7 per cent only having responded definitely to gravity.

On the other hand, when direct sunlight was reflected downward through the water, the response to gravity was increased from 77 per cent, the normal, to 85 per cent of the tadpoles.

In all cases in which abnormal conditions of light were produced, whether reflected upward or downward through the column of water, an abnormally large number of tadpoles remained floating at the surface, unattached but undergoing normal metamorphosis.

TABLE 2

Showing number and percentage of tadpoles which became attached at various levels in a cylinder when subjected to unusual light conditions

EXPERIMENT NUMBER	NUMBER OF TADPOLES USED	FLOATING	AT EDGE OF WATER SURFACE	ABOVE MIDDLE	BELOW MIDDLE	TO BOTTOM AND BOTTOM EDGE
Cylinder jacketed; light from north window reflected upward through the column of water						
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
1 and 3	179	48	30	1.6	1.1	18.9
Cylinder jacketed; light from north window reflected downward through the column of water						
2 and 4	212	33	11.7	2.8	7	35.2
Cylinder jacketed; direct sunlight reflected upward through the column of water						
5	102	31.3	55.3	0.9	0	11.7
Cylinder jacketed; direct sunlight reflected downward through the column of water						
6	92	5.4	1	6.5	1	85.8
Cylinder placed in a dark room during free-swimming and attachment periods of the tadpoles						
12	315	18.3	33.9	1.9	2.5	43.1

These results seem to show that the positive response to gravity is normally developed and most perfectly exhibited in

the presence of directive rays of light, but the fact that 43 per cent of the tadpoles in experiment 12, in which light was absent, became attached to the bottom, shows that the positive geotropic response is not entirely conditioned by the presence of light.

That the behavior of tadpoles in their normal habitat is probably not very different from that observed under laboratory conditions may be inferred from the vertical distribution of the species.

Amaroucium colonies are found in abundance on the piles of the Vineyard Haven wharf. They are most thickly set in a zone around each pile about 1 foot in width situated just below extreme low-water mark, then, for a depth of about 7 feet, colonies are scattered rather abundantly, but are seldom found on the parts of piles located more than 8 feet below low-water mark. The in-shore piles, standing in water having a depth of 8 feet or less, are set with colonies from low-water mark to bottom in the same way as the upper 8 feet of piles standing in deeper water. The reason for the absence of colonies from the lower parts of the deep water piles is not evident for, as Van Name[5] and Sumner[6] have recorded, the species is common at depths varying from 1 to 15 fathoms on the rocky, gravelly and sandy bottoms of Vineyard Sound and Buzzards Bay, and Sumner, in comparing the distribution of A. pellucidum constellatum and A. stellatum, on the basis of eighty-five dredging stations, states of the former that it "was recorded fifteen times from depths less than 5 fathoms, while in over 60 per cent of the cases it was taken at depths under 10 fathoms."

ATTACHMENT

Three adhesive papillae, by means of which the initial attachment of the Amaroucium tadpole is effected at the close of the free-swimming period, are situated at the anterior end of the body in the median sagittal plane, fig. 1. Each papilla terminates at the surface of the tunic in a cup-shaped enlargement which contains a droplet of viscid substance. Toward the close of the

free-swimming period of the tadpole a contraction of each cup-shaped enlargement takes place sufficient to extrude their viscid contents upon the outer surface of the tunic and, when these viscid droplets come into contact with the surface of some foreign body, the tadpole sticks fast. Attachment often takes place shortly before metamorphic changes begin, and a tadpole thus caught struggles actively to free itself, sometimes with success, but more often without.

When tadpoles are placed in Syracuse watch-glasses in quantities of water sufficient only to cover the bottom, less than 50 per cent are likely to become attached, because under these conditions the activities of the tadpoles do not bring the adhesive papillae into contact with the glass surface of the dish, but, if the quantity of water is sufficient to permit the tadpoles to swim against the vertical side, attachment will take place in practically all cases. Tadpoles which fail to attach to the side or bottom of dishes well filled with water and remain floating on the surface, are sometimes found to have small bubbles of air adhering to them and, in these cases, the failure to swim or sink below the surface is thus sufficiently explained, but many cases of failure to become attached cannot be so simply accounted for. The tadpoles which do not become attached, but remain floating, are not abnormal, for they undergo metamorphosis as rapidly and as normally and develop into ascidiozooids as normal as those which become attached.

In case the floating individuals happen to drift against the surface of some foreign body, they may yet find attachment by means of the new tunic material which is abundantly secreted after metamorphosis begins. When first secreted it is somewhat viscid, spreading over and adhering to the substratum.

Initial attachment may be effected by the cement substance exuded from any one of the adhesive papillae, and no indication has been found that attachment takes place more often or more effectively at one attachment point than at another. The point by which attachment will be made apparently depends upon which one first touches a foreign surface after the droplets of cement substance have been extruded from the glandular ends

of the papillae. The struggles of the tadpole after its initial attachment frequently cause the body to adhere at all three points.

DURATION OF THE FREE-SWIMMING PERIOD

The duration of the free-swimming period in the cases of one hundred tadpoles has been accurately determined with the result shown in the diagram (fig. 4). From this it may be noted

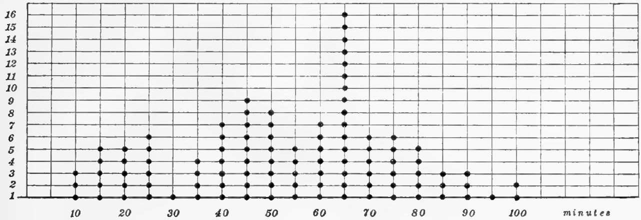

Fig. 4 Diagram showing the duration of the free-swimming period of 102 tadpoles plotted to the nearest five-minute interval.

that metamorphosis begins in some cases as early as ten minutes after the escape of the tadpole from the parent colony and that none of the tadpoles observed had an active period of longer duration than an hour and forty minutes. It should be stated, however, that in the experiments made to determine the reactions of tadpoles to light and gravity, in each of which large numbers of tadpoles were used, a few tadpoles in each case continued to swim for two hours.

CONCLUSIONS

The observations of the tadpole larva of Amaroucium recorded in this paper show:

1. That the body of the tadpole as it is propelled through the water by the tail, is in constant clockwise rotation on its long axis, the rotation being caused either as a consequence of the asymmetrical form of the body or by a torsion of the tail during its strokes or by a combination of both.

2. That tadpoles show a definite positive response to light for a very brief interval immediately following their liberation from the parent colony, but react negatively to light during the latter and greater part of the free-swimming period.

3. That tadpoles invariably remain at or near the upper surface of the water during the first part of their free-swimming period, but, as the time for metamorphosis approaches, descend into the lower strata of water and swim at or near to the bottom. This behavior has been interpreted to indicate responses to gravity, negative at first, positive finally. The positive response to gravity is exhibited in varying degrees of definiteness, not at all in a small per cent of tadpoles. Its expression seems to be aided by, but not entirely conditioned upon, the presence of directive rays of light.

4. That the viscid contents of the glandular ends of the adhesive papillae are extruded upon the outer surface of the tunic toward the close of the free-swimming period and that the initial attachment of the tadpole takes place when one or more of these protruding viscid droplets comes (accidentally) into contact with the surface of a foreign body.

5. That the free-swimming period of the tadpole varies in duration from ten minutes to two hours.

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Resumen por el autor, C. H. Edmondson.
Universidad de Oregon.

Sobre la formación de un nuevo estilete cristalino después de la extracción del primitivo en Mya arenaria.

La función del estilete cristalino de los lamelibranquios, tal cual se considera actualmente, es la de un fermento digestivo, que al ser impelido con un movimiento de rotación por la acción de las fuertes pestañas vibrátiles del saco que le contiene, penetra en el estómago donde es digerido por el contacto con el escudo gástrico. El estilete cristalino de Mya es muy resistente, presentando un ligero grado de disolución después de catorce dias de dieta. El saco del estilete está casi completamente separado del tubo digestivo, presentando a lo largo un surco manifiesto, limitado a cada lado por typhlosoles, de los cuales el derecho e stá mas marcado que el izquierdo. Las células de los typhlosoles son mas altas, de menor diámetro, y poseen pestañas vibrátiles mas cortas que las de las células que tapizan interiormente el saco. Las células de los typhlosoles aparentemente segregan mucus. Próximamente el 50 por ciento de los individuos de Mya sobreviven a la extracción del estilete cristalino. El estilete en vías de regeneración comienza a aparecer unos seis dias después de la extracción del contenido anteriormente en el saco. Al principio el estilete es un tracto de mucus arrollado, que contiene partículas alimenticias; después se arrolla hacia la derecha. El arrollamiento y las partículas alimenticias desaparecen cuando el estilete alcanza la madurez. Aparentemente los animales no ingieren ni digieren alimento normalmente hasta que el estilete es de suficiente tamaño para proyectarse en el interior del estómago. Un estilete en vías de regeneración puede aparecer en una porción del saco enteramente separada del resto. Bajo condiciones favorables la regeneración completa del estilete de Mya puede verificarse en setenta y cuatro dias.

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THE REFORMATION OF THE CRYSTALLINE STYLE IN MYA ARENARIA AFTER EXTRACTION

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THIRTY FIGURES

CONTENTS

1. INTRODUCTION

For more than two hundred years after Anton de Heide's first allusion (1686) to the 'stylus crystallinus' in his monograph on the common mussel, the significance of this peculiar organ present in certain molluscs was a matter of mere conjecture.

Nelson ('18) presented a very complete review of the many theories advanced by a host of observers in the period during which the crystalline style has been an object of attention. It is to be noted that within the past thirty-eight years, as a result of experimental work, this long list of hypotheses regarding the value of the crystalline style to those molluscs possessing it has been, by a gradual process of elimination, reduced to three, each having the support of investigators of considerable ability.

Basing conclusions upon the fact of the dissolution of the crystalline style in certain lamellibranchs during periods of starvation and hibernation, Hazay ('81), Haseloff ('88), and others have defended the theory that the organ represents reserve food

material stored up during times of excess nutrition and assimilated by the animal when needed. The comprehensive investigations of Barrois ('89–'90), Coupin ('00), Mitra ('00), and later workers have rendered the 'reserve food' hypothesis untenable. It has been pointed out that marine lamellibranchs are never without food except for short durations on the recession of the daily tides, moreover, it is well known that, in case of many marine bivalves, the temporary cutting off of the food supply by the recession of the tide is not followed by a dissolution of the crystalline style or by any marked change in it. The writer has kept Mya arenaria alive for fourteen days without food during which period only slight evidences of dissolution of the crystalline style were detected.

Coupin ('00), in a summary, says: "The chemical investigations show that the crystalline style does not contain sugars or fats and only traces of albuminoid materials."[1] The same investigator also shows that the crystalline style of Cardium edule when dried weighs but 0.004 gram, and remarks that this would seem an insignificant amount of food for an animal of this size.

After ably disposing of the 'reserve food' theory championed by Haseloff ('88), Barrois ('89–'90) concludes that the function of the crystalline style is to furnish mucus for the coating of sand grains and other foreign bodies in the stomach thereby preventing injury to the epithelium of the digestive tube as they pass through. Pelseneer ('06) follows Barrois when he says, with reference to the crystalline style: "The product of its solution forms a sort of cement which encrusts any hard substance that may have been ingested and thus protects the delicate walls of the intestine from injury." Coupin ('00), as quoted below, not only ascribes to the crystalline style a digestive function, but suggests a lubricating value when he says that the mucus of the style may agglutinate the solid particles which float in the stomach.

Schultze ('90) agrees with Barrois, drawing an analogy between the supposed function of the crystalline style and the secretion of certain glands of the larvae of batrachians which furnish a sub-

[1] My translation.

stance serving to envelop foreign materials thereby protecting the delicate mucous coat of the gills and the intestinal tract.

Kellogg ('92) raises an objection to the lubricating theory, believing that the crystalline style could not possibly serve as a coating substance for the large quantity of sand taken in by some species of lamellibranchs. Nelson ('18) has shown that the mucus which envelops foreign bodies in the stomach of the mollusc, is a product of the glands of the oesophagus, which produce a thick, heavy mucus unlike that furnished by the dissolution of the crystalline style.

That the crystalline style might function as an alimentary ferment was first suggested by de Heide in 1686. That this early conjecture represents the correct interpretation of the significance of the organ in lamellibranchs has been a growing belief among biologists during the past nineteen years. The convincing investigations of Coupin ('00), Mitra ('01), List ('02), Van Rynberk ('08), Gutheil ('11), Nelson ('18), and others have left no trace of doubt as to the true meaning of the crystalline style apparently so necessary to life and the proper functioning of lamellibranchs.

Coupin ('00) after testing the action of a solution of crystalline styles upon starch material says:

> On peut donc conclure, de ces expériences, que la tige cristalline des Acéphales est un suc digestif, une sorte de comprimé de diastases, contenant beaucoup d'amylase et un peu de sucrose, le tout noyé dans une matière muquese, laquelle a sans doute pour but d'empêcher la trop rapide dilution de la tige dans l'eau de mer contenue dans l'estomac, et peut-être aussi d'agglutiner les matières solides qui flottent dans celui-ci.

The weight of authority since 1900 is essentially in accord with Coupin's conclusion that the crystalline style functions as a digestive ferment. Mitra ('01) differed from Coupin in that he believed the crystalline style represented a solid mass of ferments. Van Rynberk ('08), by a series of experiments on the crystalline style of Mytilus, confirmed the results of Coupin and Mitra regarding the presence of an amylolitic ferment and concluded that cytases and proteases were absent. Nelson ('18) looked upon the crystalline style as a digestive ferment and has

shown that the organ rotates, as was suggested by List ('02), in the style sac in a clockwise fashion, when viewed from the anterior end, thereby serving to assist in the separation of food from foreign substances in the stomach as well as taking the place of intestinal peristalsis.

A summary of the function of the crystalline style, as recognized at the present time, is as follows: the style is pushed forward into the stomach by a movement of the strong cilia of the style sac, at the same time slowly rotating in the direction of the hands of a clock, when viewed from the anterior end. The head of the style, being in contact with the gastric shield of the wall of the stomach, is gradually worn away and, during rotation, assisted by movements of the cilia of the stomach wall, separates the refuse matter from the food, twisting the latter up into a mass of mucus at the end of the dissolving style where it may be readily acted upon by starch converting enzymes released from the style.

2. METHOD OF PROCEDURE

It is apparent that most of the work of previous investigators, with respect to the formation and function of the crystalline style, has been with those types of lamellibranchs in which there is an incomplete separation of the intestine from the caecum in which the style rests. No published reports, so far as the writer is able to determine, describe the formation of the crystalline style of molluscs in which the style sac is completely or almost completely separated from the intestinal tract.

With the hope of adding some small contribution toward the solution of this problem, the experiments, the results of which are set forth in the present paper, were initiated.

Working upon the theory that the reformation of the crystalline style, after extraction from the body of the mollusc, would take place in a normal manner if the animal were kept under natural conditions, it was decided to select a species, remove the crystalline styles from a large number of individuals, restore the animals to their normal surroundings, and by examination at more or less regular intervals follow the formation and development of the crystalline style in this particular species.

After considering the list of bivalves on our west coast, Mya arenaria, the eastern long-neck clam, was selected as being the only possible species upon which such an operation as contemplated might be successfully performed.

In the first place, Mya, by reason of its anatomical features, has proved to be exceptionally well adapted for this kind of experimental work. This favorable structural feature lies in the fact that the style sac is exposed along the ventral surface of the visceral mass for a distance of from 20 to 30 mm. in specimens ranging from medium to large size (figs. 1 to 3). Furthermore, on removing Mya from the mud it will be noticed that, in a very large number of individuals, the mantle lobes are protruding some distance between the gaping edges of the shell. The mantle lobes are fused along their anterior and ventral margins except for a small space through which the foot may be extended. It will also be found in most instances that the visceral mass of the body of the mollusc is pressed closely against the ventrally protruding mantle lobes.

By cutting the mantle in the midline along the ventral surface for a distance of from 15 to 25 mm. posterior to the pedal opening, the exposed length of the style sac is brought into view. Using fine-pointed scissors, one may easily sever the style sac transversely or longitudinally at the distal extremity or at any level in the exposed length of it. Usually immediately upon the severance of the sac the style will be forced out at the point of division. Occasionally the crystalline style will be thrown entirely out of the body by a sharp muscular contraction when the sac is clipped. More often, however, the use of fine-pointed forceps is necessary to draw the style from the sac.

In the majority of cases I have severed the style sac and style by a transverse cut at from 10 to 20 mm. from the distal extremity (fig. 3) and removed both ends of the style. The entire operation requires but a few seconds of time, and in most individuals must be accomplished immediately after the clam has been taken from the mud, as the mantle lobes are soon withdrawn and the valves of the shell tightly closed, rendering the removal of the crystalline style impossible.

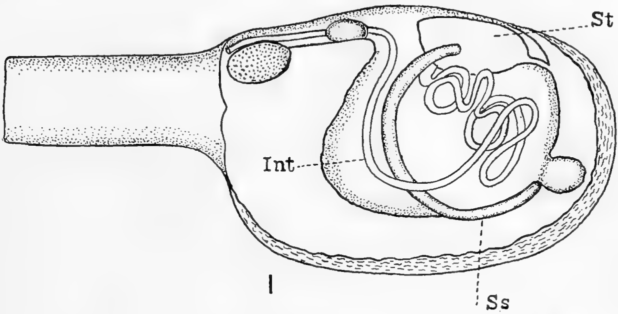
As soon as practicable after the crystalline style has been extracted, the clams should be replanted in selected areas, and each individual so marked that there may be no possibility of mistaken identity of the specimen, even though months should elapse before it is reexamined. I have used a system of stakes, by which each individual may be identified, with entire success.

The experiments on the reformation of the crystalline style of Mya arenaria were carried on in the Siuslaw River about four miles from its mouth, between the towns of Florence and Acme, where is located one of the most extensive beds of this species on the Oregon coast. Here Mya grows to a very large size, many specimens I have measured attaining a length of shell of 150 mm. The beds in the Siuslaw River are very accessible and work can be done upon them at a moderately low tide, this locality being a most convenient one in carrying on series of rapidly repeated tests or those running through longer periods of time.

The physical effect of the operation, as described above, upon the mollusc itself may be indicated by a summary of the result of the entire series of experiments. I have found that approximately 50 per cent of the clams die as a result of the severance of the style sac and the removal of the crystalline style. In some tests extending over a period of several weeks the death rate ran as high as 75 per cent, while in others, enduring for a similar period, it was as low as 25 per cent. Unless death occurs during the second or third week following the operation, the clams usually recover and regain their normal functions, the wound in the meantime having healed and the severed ends of the style sac usually being completely closed. The success is dependent largely upon the carefulness with which the incision of the style sac is made—a very deep cut destroying the surrounding visceral tissue and permitting bacterial infection. Results also show that large, mature individuals survive more readily, apparently having a greater degree of resistance than do young or half-grown ones.

In general, a higher rate of mortality was reached in experiments carried on during the winter than the summer months. However, excessively high water and frequent flooding of the

clam beds with fresh water from an adjacent tributary of the Siuslaw River for periods of several weeks had, I believe, its effect upon the death rate at this time. Moreover, during the fall and winter months Mya arenaria is not in the best physical condi-



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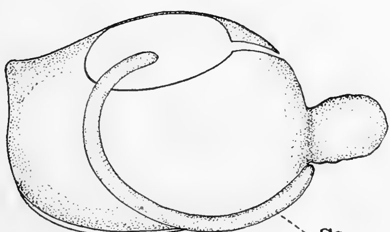

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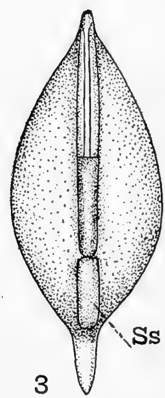

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Fig. 1 Semidiagrammatic sketch of Mya arenaria showing the relation of the crystalline style to the digestive tract. *St.* stomach; *Ss*, style sac; *Int*, intestine. $\times \frac{2}{3}$.

Fig. 2 Semidiagrammatic sketch showing the position of the crystalline style in the visceral mass. *Ss*, style sac. $\times 1\frac{1}{2}$.

Fig. 3 Ventral view of the visceral mass with the exposed portion of the style sac. The usual point of severance of the style sac is indicated by the transverse constriction. *Ss*, style sac. $\times 1\frac{1}{2}$.

tion. The spawning season of the species in this locality is in the late summer, extending into September. The energy of the animal is at a low ebb after spawning, the following months being a period of physical restoration and of increasing resistant power.

A relatively small number of animals survived when environmental modifications were brought about by restoring the molluscs, after the operation, to the sandy soil near the river shore instead of returning them to the soft, black mud of the bed from which they were taken. Although these latter experiments were conducted during the winter months, I believe the change of soil, the longer time out of water between tides, and other accompanying conditions may have contributed to the high death rate in the tests carried out along the river bank.

The experiments were commenced on February 23, 1918, and conducted continuously for twelve months, one series following another, each series consisting of from one to three dozen clams with crystalline styles removed, each group being reserved for examination at a definite interval after the operation, the intervals depending somewhat upon the condition of the tides, but ranging from six to seventy-four days. It was found that this latter time was approximately the period required for the complete reformation of the crystalline style in this species.

3. THE MATURE CRYSTALLINE STYLE IN MYA ARENARIA

Among the common marine lamellibranchs of the northwest coast great variation in the resistant character of the crystalline style may be observed. In some forms a complete dissolution of the style readily occurs if the animal is removed from its natural environment or subjected to an extended period of starvation. Among the species which may be mentioned in this connection are Cardium corbis, Saxidomus giganteus, Saxidomus nuttallii, Paphya staminea, etc. Owing to the shape of the crystalline styles of the species just mentioned, it will be found when the animals are taken out of the water and subjected to unusual conditions that the styles soon creep into the stomachs and coil themselves up there, resulting in the style sacs being empty long before the crystalline styles are actually dissolved.

The complete dissolution of the styles of these species occurs, however, within a few days at most. I have found the style of Paphya staminea to have entirely disappeared after a starvation period of forty-six hours, and the complete dissolution of the crystalline style of Saxidomus giganteus usually takes place within two or three days. Nelson ('18) has shown that in the eastern oyster, Ostrea virginica, the crystalline style disappears within an hour after the animal is uncovered by a recession of the tide and may be reformed in fifteen minutes after active feeding has commenced again.

On the other hand, crystalline styles of certain species are found to be very resistant, not readily dissolved, and persisting throughout the life of the animals even when the latter are removed from their normal surroundings or when death by starvation occurs. This latter group of bivalves includes Siliqua patula, Schizothaerus nuttallii, Macoma nasuta, Mya arenaria, and others.

Among these Mya arenaria possesses a crystalline style of a very high degree of resistance. It is certainly never dissolved during the intervals of the recession of the daily tides and presents a remarkable degree of persistence when the clam is placed under unnatural conditions. After eight days of starvation the crystalline style of Mya is still very firm except at the extremities where it has begun to soften. The end projecting into the stomach has been broken down into a jelly-like mass and the opposite extremity shows slight indications of dissolution. Mya may be kept alive, out of water and undergoing starvation, for a duration of fourteen days. At the end of this period the crystalline style shows but a slightly increased degree of dissolution over that presented after eight days of starvation. In Siliqua patula and Schizothaerus nuttallii the crystalline style is found to persist throughout the life of the individual, while subjected to starvation, without much noticeable alteration in its substance. The tenacity of life of these species, however, is not so great as that of Mya arenaria.

In the forms just mentioned, showing crystalline styles with great power of resistance, the style sacs are completely or, as in case

of Mya arenaria, almost completely separated from the intestinal tract.

I have not observed spirochaetes in the styles of any forms in which the style sacs are separated from the intestinal tracts, although the parasites are at times abundant in the stomachs and intestines of some of these clams, especially Schizothaerus nuttallii and Mya arenaria. I have commonly found spirochaetes in the crystalline styles of Saxidomus giganteus and Paphya staminea, in both of which species the style sac is connected with the intestinal tract throughout the length of the former. The presence or absence of these free-moving parasites in the crystalline style also assists, I believe, in establishing the relative consistency of this organ in different species of lamellibranchs.

The mature crystalline style of Mya arenaria (fig. 4) is cylindrical, becoming slightly thicker toward the extremity which projects into the stomach, which I shall designate as the proximal end, tapering gradually toward the rounded distal extremity which rests against the base of the foot of the clam. In its course from the base of the foot of the clam to the stomach the style approximates, in an imperfect manner, the arc of a circle (figs. 1 and 2). On the ventral surface of the visceral mass, for a distance of from 20 to 30 mm. in a medium-sized individual, the distal portion of the style sac is exposed to view in the midline. From this exposed region, as the crystalline style disappears into the visceral mass, it inclines to the left of the midline in its course toward the stomach entering that organ through its posterior ventral wall.

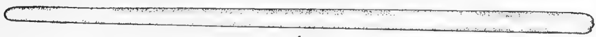
The size of the crystalline style of Mya is dependent, in general, upon the size of the animal possessing it. During digestion the proximal or stomach end of the style is worn away more or

Fig. 4 Mature crystalline style of Mya arenaria. $\times 2\frac{1}{4}$.

Fig. 5 Transverse section of the crystalline style showing the concentric layers and spindle-shaped masses of mucus. *Mc*, mucus masses. $\times 22$.

Fig. 6 Longitudinal section of a portion of the crystalline style, greatly enlarged. *Mc*, spindle-shaped masses of mucus.

Fig. 7 Horizontal section through the floor of the stomach showing the beginning of the style sac and the intestine. *St*, stomach cavity; *Ss*, style sac; *Gs*, gastric shield; *Int*, intestine. $\times 25$.



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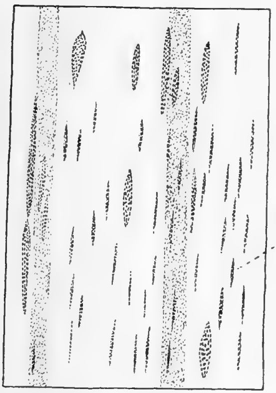

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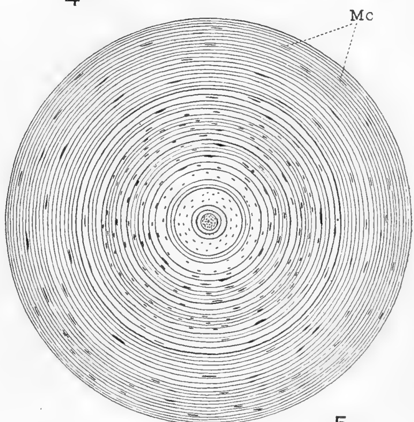

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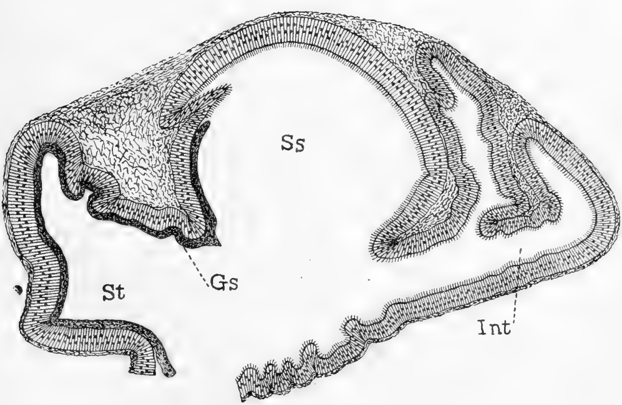

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less rapidly. Nelson ('18) found that the rotary movement of the crystalline style in Modiolus was not continuous during digestion, but was inhibited at intervals, periods of rest alternating with periods of activity. Repeated attempts to verify the rotary movements of the crystalline style in Mya have so far been without success on my part, but from observations on the development of the organ, as presented later in this paper, one may safely conclude that rotation occurs.

By comparing the lengths of the crystalline styles in a large number of individuals with the shells, one finds that the style of Mya arenaria averages approximately 72 per cent of the length of the shell. In a specimen with a shell length of 120 mm. the crystalline style usually measures from 85 to 90 mm. In diameter the style of a medium-sized specimen of Mya is approximately 3 mm. at the larger extremity. The style is firm, quite solid, and translucent in a fresh specimen, but lacks the luster so characteristic of the same organ in Schizothaerus nuttallii and Macoma nasuta.

Cross-sections of the crystalline style of Mya arenaria reveal the concentric layers in the substance of the organ (fig. 5). I have been able to count from eighty to one hundred of such layers, which vary considerably in thickness.

At irregular intervals between the concentric layers of the crystalline style of Mya arenaria I have been able to make out minute, spindle-shaped areas which have the morphological appearance and the staining reaction of mucus. These are represented in a portion of a longitudinal section under high magnification (fig. 6). I have at no time observed a central core of food matter in a mature crystalline style of Mya arenaria.

4. THE RELATION OF THE CRYSTALLINE STYLE TO THE INTESTINAL TRACT IN MYA ARENARIA, AND FEATURES OF THE STYLE SAC

In Mya arenaria, for a distance of from 10 to 15 mm. from the floor of the stomach, the style sac and the proximal extremity of the intestine are incompletely separated (figs. 1 and 17). Cross-sections through the style sac and intestine just below the floor of the stomach show the union between the two tubes, in all es-

sential features, to be similar to the union of the intestine and the style sac existing throughout the entire length of the latter in Cardium corbis, Saxidomus giganteus, Paphya staminea, and others. In these cross-sections prominent typhlosoles are observed to be developed, one on either side, separating the intestinal tube from the style sac (figs. 7 and 8). Here the intestine is on the anterior surface of the style sac, and I have designated one typhlosole as the left and the opposite one the right.

After being in union for a short distance below the floor of stomach, the intestine breaks away from the anterior border of the style sac, making a sharp turn toward the stomach wall, bending ventrally again and beginning the characteristic series of loops in the visceral mass (figs. 1 and 17). The intestine crosses to the right of the crystalline style as it ascends toward the dorsal border to traverse the pericardial cavity.

At the point of separation of the intestine from the style sac, and as a result of this separation, an evagination of the style sac occurs (fig. 9). The groove thus formed continues on the antero-lateral border of the tube, becoming less prominent toward the distal end, and fades away as a distinct groove among the numerous folds of the inner wall at this extremity.

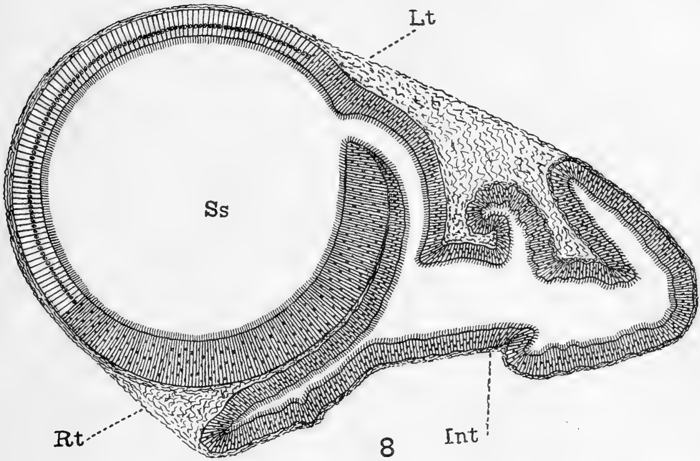
Transverse sections at the point of separation of the two tubes clearly indicate that the groove is a remnant of the intestinal tract formed by the latter as it is drawn away from the style sac. In the formation of this groove in Mya arenaria the evidence points toward this species being a transitional form between lamellibranchs in which the style sac is united throughout its length with the intestinal tract, and those species in which the intestine and the style sac are completely separated. The cells lining the groove have the same general characteristics as the cells of the epithelium of the intestinal tube. In the bottom of the groove thus formed the cells are short, becoming longer on the sides where they merge into the epithelium of the style sac proper. The position and arrangement of the nuclei of cells of the groove vary somewhat, due to modification of the cells in the different regions of it, but the nuclear arrangement characteristic of the epithelium of the digestive tube is generally

maintained. Cilia similar to those of the intestine cover the free ends of the cells of the groove.

The typhlosoles separating the style sac from the intestinal tract near the stomach wall now become the typhlosoles separating the groove from the style sac proper and maintain their relative position and importance, the more prominent one being on the right side.

Histological preparations of the style sac, by cross-sections through the region of its union with the intestine, give a view of the cells covering the typhlosoles (fig. 8). On the right typhlosole, extending nearly one-third the circumference of the tube, the cells are very long, narrow, so closely crowded together that their nuclei have become compressed and are situated at different levels. The free ends of the cells are provided with a dense layer of cilia somewhat shorter than those covering the cells of the general surface of the style sac. The opposite typhlosole may lack entirely or carry but a small group of these long, narrow cells. The epithelium of the general lining of the style sac consists of cells characterized by their uniformity of size, being approximately 0.13 mm. in length and 0.016 mm. in diameter in a medium-sized individual (fig. 14). In these cells the nuclei are uniformly oval, each with a distinct nucleolus, and are located near the middle region of the cells all approximately at the same level. A basement membrane resting upon a thin layer of loose connective tissue supports the cells. Attached to the free ends of these cells is a very dense layer of strong cilia slightly longer than those carried by the cells of the typhlosoles. In a medium-sized individual the cilia of this dense layer measure about 0.04 mm. in length or one-third the length of the cells which bear them.

The cytoplasm of the cells just described is granular and often their free extremities are crowded with minute granules of brown pigment. Sabatier ('77) concludes that this brown, granular pigment, characteristic of the cells of the general surface of the style sac, is derived from the substance of diatoms, protozoa, etc., collected between the crystalline style and the epithelium of the lining of the tube and squeezed out by the action of the strong cilia. This involves the function of the crystalline style as pro-



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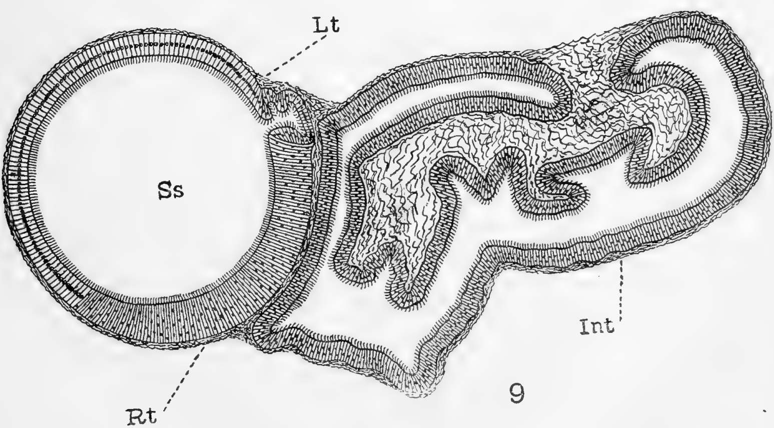

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Fig. 8 Transverse section through the style sac and intestinal tract 5 mm. below the floor of the stomach. *Ss*, style sac; *Int*, intestine; *Ls*, left typhlosole; *Rt*, right typhlosole. × 38.

Fig. 9 Transverse section through the style sac and intestinal tract just below the point of separation of the two tubes. *Ss*, style sac; *Int*, intestine; *Lt*, left typhlosole; *Rt*, right typhlosole. × 25.

posed by that investigator, namely, that the organ serves as a crushing surface for food collected between it and the epithelium of the style sac. As there is no means by which food may enter the style sac in species in which the style rests in a distinct caecum, the opinion of Sabatier is not well founded. I have observed the brown pigment in Mya to be most abundant during the winter months and the cells nearest the typhlosoles are more densely crowded with it. In cells remote from the typhlosoles it is barely perceptible or may be entirely lacking. Since the typhlosoles are highly vascular, I am of the belief that the pigment may reach the cells from the circulatory system.

Cross-sections of the middle and lower third regions of the style sac indicate a gradual lessening in extent of the narrow cells of the right typhlosole (fig. 10). The groove becomes more shallow and the typhlosoles less prominent as the distal extremity of the style sac is approached. At the very distal tip of the style sac transverse sections show the epithelium of the lining to be greatly folded, while the groove and typhlosoles have lost their identity (fig. 11). Longitudinal sections of the style sac represent the epithelium of the general surface of the tube to be thrown into transverse folds which are low undulations in the proximal portion of the tube, but become stronger and more irregular ridges toward the distal extremity (figs. 12 and 13).

Fig. 10 Transverse section of the style sac through the middle region. *Ss*, style sac; *G*, groove of style sac; *Lt*, left typhlosole; *Rt*, right typhlosole. × 25.

Fig. 11 Transverse section through the distal extremity of the style sac showing the folding of its inner wall. × 25.

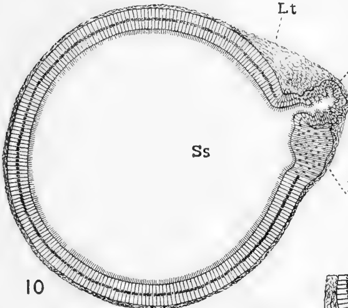
Fig. 12 Longitudinal section of the style sac in its proximal half. *Bm*, basement membrane; *C*, cilia. × 120.

Fig. 13 Longitudinal section of the distal half of the style sac showing the increased size of the transverse folds of the epithelium over those of the preceding figure. *Bm*, basement membrane; *Tr*, transverse folds of the epithelium of the style sac. × 75.

Fig. 14 A section of a group of cells of the general surface of the style sac. × 280.

Fig. 15 The gastric shield after removal from the left wall of Mya arenaria. × 7.

Fig. 16 A transverse section of a fold of the left wall of the stomach to which is closely applied the arms of the gastric shield; showing also the stratified nature of the gastric shield. *Gs*, gastric shield. × 23.



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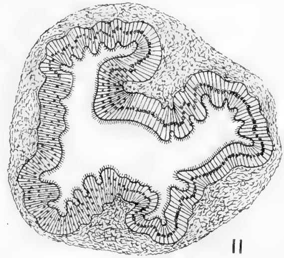
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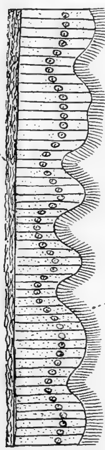

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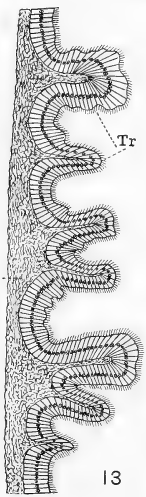

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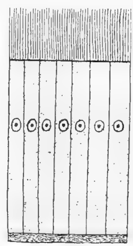
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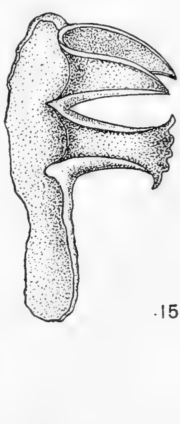
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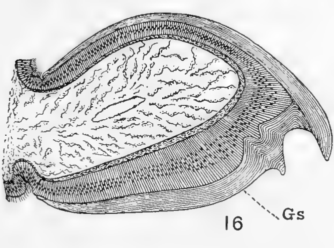

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The circular folds of the style sac do not continue to the margin of the groove, but abruptly terminate at the lateral border of the long, narrow cells on either side of the groove. Therefore the typhlosoles are not marked by undulating epithelium as is the rest of the tube, but are smooth on their inner surfaces. This latter feature is made plain by longitudinal sections, close to the margin of the groove, in which the epithelium is seen to rest upon a basement membrane disposed in an even line without folds or undulations.

5. THE GASTRIC SHIELD IN MYA ARENARIA

Spreading over the left wall, the roof, and the floor of the posterior half of the stomach of Mya arenaria is found the structure which Nelson ('18) called the gastric shield. In this species the shield does not cover the right wall of the stomach (fig. 18).

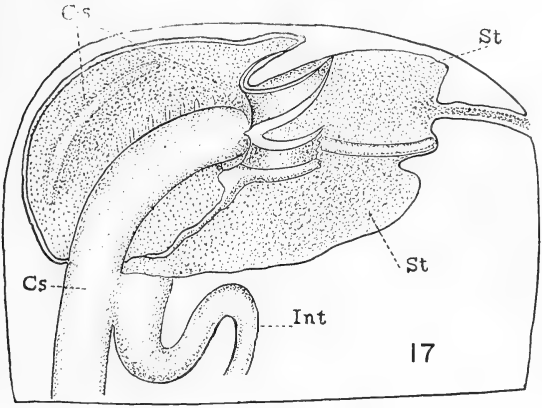
The gastric shield consists of a cartilage-like layer applied to the epithelium of the stomach wall growing thicker from the posterior region forward where it develops into an irregular structure with three curved processes which clasp prominent folds of the dorsolateral wall of the stomach (figs. 15, 16, and 19).

Poli (1791) first described this organ under the name of 'fleche tricuspide,' and suggested that it might control the flow of bile into the stomach by the extension of its processes into the bile crypts.

Histological sections through the stomach wall and gastric shield of Mya arenaria show the latter to be closely applied to the columnar epithelium. Beneath the shield the epithelial cells are not provided with cilia. It will also be observed that the gastric shield consists of numerous strata comparable to the concentric layers of the crystalline style in cross-section.

Fig. 17 Semidiagrammatic section showing the relation of the crystalline style to the gastric shield in Mya arenaria and also to the proximal extremity of the intestine. *St*, stomach; *Gs*, gastric shield; *Cs*, crystalline style; *Int*, intestine. × 2.

Fig. 18 A transverse section of the stomach of Mya arenaria showing the position of the gastric shield on the roof and the left wall of the stomach. *St*, stomach; *Gs*, gastric shield; *Ss*, style sac. × 3½.



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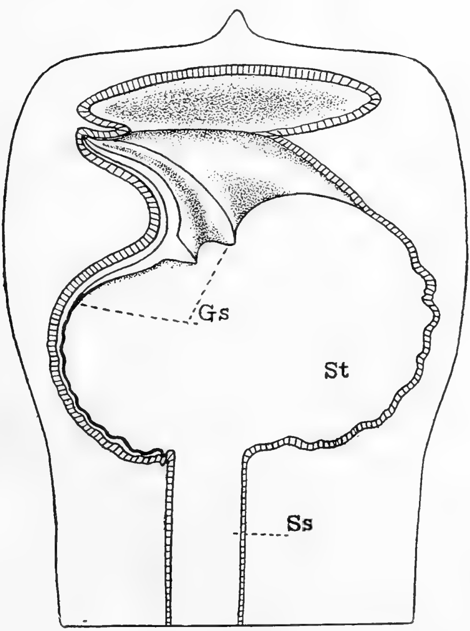

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According to Gutheil ('11), the gastric shield is built up by a hardening of the material secreted by the epithelium on which it rests. Nelson ('18) states that it is probably in the nature of chondrin. In Mya arenaria the structure is very resistant, slight dissolution of its thickest region occurring during a period of about thirty days after the removal of the crystalline style.

This peculiar structure, without doubt, serves as a protection to the epithelium of the wall of the stomach which it covers, and at the same time it assists in the wearing away of the end of the crystalline style which, in its rotation, presses against the concave surface of the thickened portion of the shield (fig. 17).

6. DETAILS OF THE EXPERIMENTS

In presenting the details of the various experiments carried on for the determination of the reformation of the crystalline style, following its extraction, in Mya arenaria, it is my purpose to consider the series of experiments in the order of the progressive development of the crystalline style rather than the chronological order in which the experiments were conducted.

Experiment 1. September 19, 1918

Six medium-sized clams, with crystalline styles removed by transverse sectioning of the style sacs about 10 mm. from the distal extremities, were replanted under normal conditions. The experiment was one of short duration, continuing for six days, with the object in view of determining the exact character and position of the crystalline style in its initial stage.

Result, September 25, 1918. After six days all of the clams operated upon were found to be alive and in good condition. The severed ends of the style sac were healed in each individual, and in one or two speci-

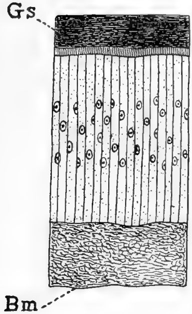
Fig. 19 A section of the left wall of the stomach of Mya arenaria with the gastric shield closely applied to the inner surface of the epithelium. *Bm*, basement membrane; *Gs*, gastric shield. × 84.

Fig. 20 The regenerating crystalline style four days after extraction, the clams being out of water and without food but subjected to low temperature. × 2½.

Fig. 21 The crystalline style after a growth of six days under normal conditions. × 2½.

Fig. 22 The same as the preceding figure. × 2.

Fig. 23 A regenerating crystalline style thirteen days after extraction. × 2.



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mens were closed. In each of the animals the short, distal portion of the style sac, separated from the remainder by the operation, was filled with mud and other foreign material.

In each of five of the specimens, on opening the long, proximal section of the style sac by a dorsal, longitudinal incision, the beginning of a crystalline style was evident. At this early stage the crystalline style consisted of a delicate mucilaginous sheath enclosing an axillary core of food and foreign material which included diatoms, sand particles, spicules, etc. The delicate style at this period occupied the entire length of the sectioned style sac and was greatly convoluted and beginning to coil (figs. 21 and 22). In three specimens the style was closely applied to the right typhlosole; in one animal it occupied the left typhlosole, and in one it alternately lay on the right typhlosole and in the groove throughout its length.

The diameter of the crystalline style at this stage of its development averaged about 0.3 mm., but varied at different levels due to the food and foreign material being massed in the axis in greater amounts at some points than at others.

Apparently the digestive processes were inhibited in the clam during the interval of six days, the stomach being empty and the intestinal tract entirely free of food material. No change was observed in the gastric shield of the wall of the stomach at the close of this period.

Experiment 2. November 16, 1918

Eight clams, each with the crystalline style removed by a transverse severance of the style sac near the distal extremity, were replanted under normal conditions. The experiment extended over a period of thirteen days.

Result, November 29, 1918. Five of the eight clams were found to be alive and in good condition. The severed ends of the style sac were healed in each surviving specimen, and in one or two animals were entirely closed. In each individual the short, blind end of the style sac was filled with mud, while the proximal division was occupied by a crystalline style in the course of development. At this period the crystalline style presented a considerable degree of advancement over one of six days' growth. It was tightly coiled throughout its length, being twisted to the right when viewed from the proximal end. A food core occupied the central axis of the style (fig. 23).

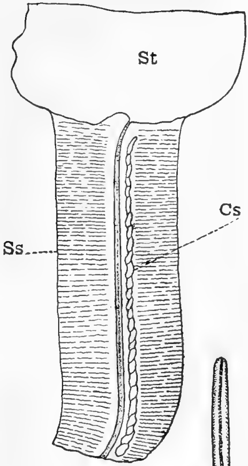
Fig. 24 A sketch of the style sac opened along the posterior border showing the crystalline style of thirteen days growth lying on the right typhlosole. *St*, stomach; *Ss*, style sac; *Cs*, crystalline style. Natural size.

Fig. 25 The crystalline style thirty-four days after extraction. × 2.

Fig. 26 A reformed crystalline style fifty-two days after extraction. × 2.

Fig. 27 A crystalline style sixty-nine days after extraction. × 1½.

Figs. 28–30 Crystalline styles approaching maturity, seventy-four days after extraction. × 1½. Some crystalline styles were completely reformed after this period of regeneration.



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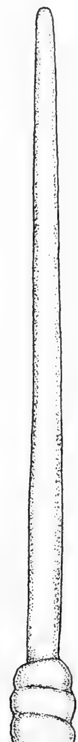

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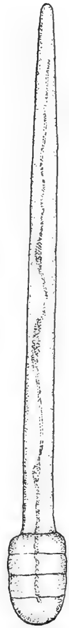

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In each individual the organ lay on the right typhlosole, occupying nearly the entire length of the tube, but did not protrude into the stomach (fig. 24). In diameter the style measured 0.5 mm. at the distal extremity, which at this time was somewhat thicker than the proximal end. That no food had been taken by the animal during the interval of thirteen days seemed apparent by the empty and blanched condition of the intestines. The stomach was without food in each surviving individual.

The material forming the food core of the crystalline style at this early period of its development is probably furnished from that which was in the stomach at the time of the extraction of the style. Although the style sac is an open tube leading from the stomach after the removal of the crystalline style, in no specimen examined have I been able to detect a particle of food or foreign material being carried down this tube from the stomach except that enclosed by the style itself.

Experiment 3. February 23, 1918

Twelve clams, ranging in size from 40 to 125 mm., with styles removed, as in the preceding experiment, were planted under normal conditions. This test extended for a period of sixteen days.

Result, March 9, 1918. On this date nine of the twelve clams were surviving and apparently in good condition. The cut surfaces were well healed, and in a number of specimens the style sacs were also closed at the severed ends. On opening the proximal portion of the style sac of each of these clams no appearance of a regenerating crystalline style was evident. The tube was free of foreign material, but apparently the reformation of the crystalline style had not yet made its beginning. An examination of the stomach revealed the absence of food, and the intestinal tract was also empty. It was quite evident that the clams had not been feeding since the removal of the crystalline styles. This experiment resulted negatively in so far as the reformation of the crystalline style is concerned, but, I believe, is evidence of the slow development of this organ during the winter months.

Experiment 4. November 30, 1918

Twelve clams with crystalline styles removed were taken from their native bed and planted in a sandy locality near the bank of the river where they would be exposed for a much longer period than usual by the recession of the tides. The experiment continued for twenty-six days.

Result, December 26, 1918. Of the twelve clams planted in the sandy soil near the shore line four were alive after a period of twenty-six days. Of these survivors two specimens showed no evidences of the reformation of crystalline styles. The other two possessed styles very rudimentary in development, correspondng closely to those of six days' growth as recorded in experiment 1.

The stomachs and intestines of these animals were empty, the latter exhibiting a blanched appearance indicative of the lack of activity for a considerable length of time. It was evident that the ingestion of food had been completely inhibited during the entire twenty-six days. All surviving animals were in a very weakened condition.

Experiment 5. December 27, 1918

Thirty-seven clams with styles removed as in previous experiments were returned to the natural bed. This test extended over a period of thirty-four days.

Result, January 30, 1919. Five clams survived the operation for thirty-four days. On examining these animals it was found that each had reformed a very rudimentary crystalline style. In each case the organ rested on the right typhlosole, occupying nearly the entire length of the proximal division of the style sac, but not projecting into the stomach. It was convoluted and coiled throughout its course (fig. 25). A delicate food core formed the axis of the rudimentary style. During the period of thirty-four days these crystalline styles had reached a stage of development in all respects comparable to those formed between September 19, and September 25, or during a period of six days.

No food was found in the stomach or intestines of any of these clams surviving the period of thirty-four days. The condition of the intestinal tube was as has been described in the preceding experiment, and the animals were in a very weakened condition.

Experiment 6. September 25, 1918

Twelve clams with crystalline styles removed as in preceding experiments were replaced under normal conditions. The experiment extended over a period of fifty-two days.

Result, November 16, 1918. Four of the clams were alive and apparently in good condition on this date. A crystalline style was reformed and fairly well developed in each of the surviving animals (fig. 26). The style occupied the entire length of the proximal division of the style sac and projected well into the stomach, although not in actual contact with the gastric shield. Beginning with the distal extremity, about two-thirds of the length of the crystalline style was coiled to the right when viewed from the proximal end, while the proximal third was smooth. The short, blind division of the style sac in each of the animals was closed and either filled with mud or empty.

Both the stomach and intestines in each of these clams were well filled with food, indicating the functioning of the digestive organs in a normal manner.

Allen ('14), with reference to the feeding habits of molluscs, concludes: "that appetite fulfills its function by the control of

the secretion of the digestive juices without the voluntary regulation of the food supply." The same investigator determined that the molluscs continued to feed regardless of appetite, and with regard to the crystalline style this observation was made: "The crystalline style was found to disappear only with the lack of food, and to be regenerated only when food was supplied, regardless of time."

My experiments with Mya arenaria seem to show that the ingestion and digestion of food is dependent upon the degree of development of the crystalline style, since not until this organ is sufficiently mature to be projected into the stomach, where the ferments which it contains may be of service, does the animal ingest food. The appetite is wanting until the crystalline style is functional.

Experiment 7. April 27, 1918

Twenty-four clams with crystalline styles removed as in previous experiments were planted under normal conditions. The test extended for a period of sixty-nine days.

Result, July 5, 1918. Ten of the clams operated upon April 27 were alive and in good condition. The severed style sacs were healed, and in nearly all of the specimens the cut ends were closed. In each surviving individual the proximal division of the style sac contained a crystalline style nearing a state of complete development (fig. 27). The crystalline style in each case occupied the entire length of the tube and projected into the stomach, but was not in actual contact with the gastric shield. For a short distance from the distal end the style was closely coiled to the right, the remaining portion of the surface being smooth. The distal extremity was slightly larger in diameter at this period of development. Some of the crystalline styles enclosed twisted cores of food material.

In the majority of the specimens of this experiment the short, distal division of the style sac was entirely closed, forming a completely isolated tube blind at both ends. These blind tubes were usually filled with mud which collected in them before the severed ends healed, but in one individual, on opening this blind portion of the style sac cut off from the rest by the operation, it was found to be occupied not by foreign material, as in case of the others, but by a soft, gelatinous rod, extending the full length of the tube, which in all respects resembled the substance of the regenerated crystalline style of the other division of the tube.

Here was a crystalline style developing in a portion of the style sac entirely removed from any relation to the digestive tract, which would

seem to prove conclusively that the crystalline style is formed in the caecum in which it rests.

That each of the surviving clams had been feeding and carrying on the digestive processes for some time was evidenced by the presence of food in the stomach and waste material throughout the intestinal tract.

Experiment 8. July 9, 1918

Twelve clams with crystalline styles removed were returned to their natural environment. The experiment extended over a period of seventy-two days.

This experiment, with the two following ones, was conducted in order to determine, if possible, the exact time required for the complete reformation of the crystalline style in this species.

Result, September 19, 1918. Nine clams survived the operation for a period of seventy-two days, all being at the end of the period in good condition. On opening the style sac proximal of the point of severance it was found in each case that a crystalline style, well formed and almost mature, occupied the entire tube and, projecting into the stomach, was in actual contact with the gastric shield. In some specimens the crystalline style was closely coiled for a distance of about 10 mm. from the distal extremity, with the rest of the surface smooth, while in other individuals the style was enlarged for a few millimeters from the distal extremity and abruptly narrowed down to a smaller diameter. This thickening of the distal portion of the crystalline style at this stage in its development seems to indicate that material is added to its surface more rapidly in this region.

In some of the crystalline styles central cores of food material were evident. Digestive processes were being carried on apparently in a normal condition with the stomach and intestinal tract well filled with food and foreign material.

In all of the surviving specimens the distal, short divisions of the style sacs, separated by the operations, were either filled with mud or were entirely empty, no evidence of the regeneration of material comparable to crystalline style substance being found.

Experiment 9. July 8, 1918

Twenty-six clams with crystalline styles removed as in former experiments were replanted in the natural bed from which they were taken. The test covered a period of seventy-three days.

Result, September 19, 1918. Fourteen clams survived the period of seventy-three days and were in good condition on this date. The crystalline styles in all of these specimens were well developed and in most cases almost mature. The essential characteristics of the crystalline styles of this stage of development were outlined under experiment 8, there being considerable variation, but no uniform advance in maturity over those of seventy-two days.

Digestion in all of these animals was proceeding in a normal manner.

Experiment 10. July 6, 1918

Twenty-four clams with crystalline styles removed were replanted under normal conditions in the natural bed from which they were taken. The experiment extended over a period of seventy-four days.

Result, September 18, 1918. Fifteen of the twenty-four clams survived the operation and were apparently in normal condition. The proximal division of the style sac of each of these clams contained a well-developed crystalline style either completely or nearly completely reformed (figs. 28 to 30). The crystalline styles approaching maturity have been described under experiment 8. One mature style was found among the fifteen, it being identical in shape, diameter, and general appearance with a style from another animal of similar size that had not been operated upon. The regenerated crystalline styles are uniformly shorter, after the operations, than were the original ones due to division of the style sacs.

It is shown, therefore, that the crystalline style of Mya arenaria may, under most favorable seasonal and other environmental conditions, be completely reformed during an interval of seventy-four days.

In fourteen of the clams, in this experiment, the short, distal portions of the style sacs, now healed and closed, were filled with mud and foreign material or entirely empty. In one specimen this blind tube was occupied by solidified, gelatinous material, identical with the substance of the regenerated style. A similar result was obtained in experiment 7, as recorded above.

Experiment 11. Carried on during the latter part of December, 1918, and the first part of January, 1919

The crystalline styles were removed from fifteen clams, and instead of returning them to the natural bed they were placed in a refrigerator under a low degree of temperature to be examined at intervals for the reformation of the crystalline style. As has been pointed out above, Mya arenaria may be kept alive out of water, but under low temperature, for a period of approximately fourteen days.

On examining specimens subjected to the above abnormal conditions of starvation under low temperature for a period of four days, it was found that the severed ends of the style sacs were healed but not closed. By splitting open the posterior border of the style sac the newly formed style was observed as a delicate sheath of mucus, twisted and convoluted, enclosing a thread of food material, lying on the right typhlosole or, as in one specimen, applied against the left typhlosole (fig. 20). Individuals examined after a period of six days' starvation and at intervals up to the fourteenth day indicated that the development of the crystalline style, under these conditions, was practically inhibited after about the fourth day.

That the extraction of the crystalline style does not affect the tenacity of life of the animal when out of water is shown by the fact that I have been unable to keep normal individuals alive under low temperature for a longer period than fourteen days. That Mya arenaria may, however, exist for a longer period than fourteen days, without taking food, when under normal conditions, has previously been shown by experiments 3, 4, and 5.

7. THE SOURCE OF THE CRYSTALLINE STYLE

The source of the crystalline style and the manner of its formation have been subjects of conjecture and considerable controversy even among those to whom the digestive function was acceptable. To consider the stomach the source of the crystalline style was the logical result of the 'reserve food' theory. This, however, failed to explain the concentric layers as observed in sections of the organ. That the crystalline style is formed by secretions of the liver gland, that it is a product of the cells of the wall of the stomach, that it is secreted by the epithelium of the style sac itself, have each had its supporters.

Mitra ('01), concluding as did Coupin ('00) that the crystalline style contained digestive ferments, believed, however, after finding granules in the substance of the style similar to those of the liver, that this gland must be the source of the organ.

List ('02), Gutheil ('11), and Nelson ('18), after extensive work, concluded that the crystalline style was a product of the style sac, the typhlosoles furnishing the material of secretion entering into its formation.

In connection with the source of the crystalline style, the function of the different types of cells lining the style sac has been the subject of many speculations and theories. Respecting the cells of the general surface of the style sac in Mytilus edulis, Sabatier ('77) says: "The particular aspect of these cells, their richness in granular protoplasm, leads me to consider them especially as an epithelium of secretion destined to furnish a digestive fluid to the stomach."[2] He adds, "The length and force of

[2] My translation.

the cilia of this layer are to be regarded as the means of proper stomach trituration to assist the action of the gastric juice."[2]

List ('02) expressed his belief, after observing the formation of the crystalline style numerous times, that the long narrow cells of the typhlosoles were the source of the organ. In regard to the shorter cells of the general lining of the style sac, List states his opinion in the following words:

> Seine Function beruht nach meiner Ansicht vornehmlich darin, den Krystallstiel in eine drehende Bewegung zu versetzen, wodurch einerseits der regelmässige concentrische Schichtenbau bedingt wird und andrerseits ein stetiger Nachschieben in den Magen.

The assumption of List, as indicated by the above quotation, that the strong cilia of the shorter cells cause the crystalline style to rotate and to be gradually projected into the stomach, was entirely verified by Nelson ('18), who actually observed the rotation and the movement of the crystalline style into the stomach in Modiolus.

Biedermann ('10–'11) doubts that ciliated epithelium can have a secreting function, and is inclined toward Mitra's view that the crystalline style represents in reality a condensed product of the secretion formed by the liver.

Gutheil ('11), in answer to Biedermann, contends that there is no difference between secretion and absorption except in the direction of movement of the material, and points out that the intestinal tract is ciliated throughout and is an absorbing surface, therefore, ciliated epithelial cells may also secrete. Nelson ('18), from observations on species with the style sac incompletely separated from the intestine, concluded that the cells of the typhlosoles were the source of the crystalline style.

The chief morphological characteristics of the cells of the style sac of Mya arenaria have been mentioned above. On the right border of the groove the long, narrow cells, with nuclei at various levels and which Sabatier ('77) termed the white epithelium, are sharply contrasted with and abruptly merge into the general lining epithelium of the style sac, as has been described above.

Transverse sections of the style sac together with the intestinal tube just below the stomach floor, stained in mucicarmine, re-

veal the intestinal wall abundantly supplied with spindle-shaped masses of mucus. These may be traced to the summit of each typhlosole, but are not observed as differentiated masses in the epithelium of the style sac proper. The group of narrow cells of the right typhlosole, under the action of the above stain, gives a solid mucus reaction, while the general epithelium of the style sac gives no mucus reaction whatsoever.

In cross-sections through the middle region of the style sac stained in mucicarmine, a solid mucus reaction is noted in the group of narrow cells on the border of the right typhlosole. Fusiform clumps of mucus are observed in the groove, but the cells of the general surface of the style sac give no mucus reaction.

Transverse sections at different levels, stained in toluidin blue, also indicate an abundance of mucus in the right typhlosole, in the groove, and at the apex of the left typhlosole. No mucus reaction is obtained with this stain from the epithelium of the general surface of the style sac.

Comparing sections of the style sac of Mya arenaria and Schizothaerus nuttallii, it will be found that the latter, stained in Delafield's hematoxylin, show the groove and both typhlosoles to be filled with spindle-shaped masses of mucus, while scattering mucus glands are observed among the shorter epithelial cells of the tube. I have not been able to secure such a reaction in Mya arenaria treated with hematoxylin.

It would seem evident, therefore, from the work of previous investigators and from the actual observation of the position of the newly formed crystalline style, supported by histological evidence, that the typhlosoles supply the material for the substance of this organ, and that the shorter cells with strong cilia assist in molding the crystalline style into its cylindrical form and at the same time rotate it and push it into the stomach.

8. SUMMARY AND CONCLUSIONS

1. In Mya arenaria approximately 50 per cent of the individuals survive the extraction of the crystalline style by a severance of the style sac.

2. After the extraction of the crystalline style of Mya arenaria, a new style is competely reformed in the proximal portion of the style sac in a period of about seventy-four days, when conditions are most favorable.

3. At the end of the fourth day after the extraction of the crystalline style, the beginning of a new style may be observed in the style sac. It consists of a delicate sheath of mucus enclosing a core of food material and lies on one of the typhlosoles, usually the right which, in Mya arenaria, is the most prominent.

4. Growth of the crystalline style progresses more rapidly during the summer than the winter months. The increasing vitality of the mollusc preceding the spawning period, which occurs late in August and early September, may be accountable, in part at least, for the more rapid development of the crystalline style during the spring and summer. Climatic conditions are also more favorable for growth during the summer season.

5. A crystalline style is reformed, under favorable conditions, in the short, distal portion of the style sac which is entirely separated from the proximal division by the operation. This observation proves conclusively that the epithelium of the style sac is the source of the crystalline style.

6. In the reformation of the crystalline style of Mya arenaria an axillary food core is usually present, but disappears with the complete development of the organ by the gradual movement of the latter into the stomach. No food core exists in the fully developed crystalline style of this species.

7. After the removal of the crystalline style, Mya arenaria may exist for more than thirty days without ingesting food, if replanted under normal conditions, during which time the crystalline style is reforming.

The ingestion and digestion of food is apparently dependent upon the degree of development of the crystalline style, since not until the organ reaches a state of sufficient maturity to be projected into the stomach does the ingestion of food occur.

8. Mya arenaria may be kept alive out of water and without food for fourteen days after the extraction of the crystalline style. Under these conditions a new crystalline style makes its appear-

ance about the fourth day, but its development is inhibited, on account of the decreasing vitality of the animal, and progresses during the fourteen days but slightly beyond its initial stage.

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Resumen por el autor, Charles Zeleny.
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Un cambio en el gene 'bar'[1] en Drosophila melanogaster, con disminución del número de facetas y aumento de dominancia.

Durante la selección de individuos con un número bajo de facetas oculares, en moscas que poseen ojos blancos en forma de banda, apareció un solo macho cuyo ojo presentaba solamente 19 facetas, en vez del número medio 75.6 presente en los demás machos de la misma generación. Cuando se cruzó este macho con hembras de su misma generación produjo, en la tercera generación de híbridos, una raza cuyo número medio de facetas es 23. Esta raza se ha conservado sin cambiar durante dos años, y el autor propone para ella el nombre de 'ultra-bar.' La mutación mencionada es interesante porque tiene lugar en la misma dirección que la mutación original que transformó el ojo completo en ojo en forma de banda, y es un cambio en el mismo gene, mientras que otras mutaciones que afectan al mismo carácter, exceptuando la reversión a ojo completo, se deben a factores accesorios. Además dicha mutación ha aparecido en la misma dirección de la selección verificada por el autor. Finalmente, presenta una dominancia considerablemente aumentada, de tal modo que no solo es dominante sobre el ojo completo sino también sobre el ojo en forma de banda.

[1]Con este nombre se designa una mutación en la cual la porción pigmentada del ojo aparece en forma de banda vertical. Nos parece oportuno conservar la palabra original inglesa con el fín de no cambiar los símbolos empleados en las fórmulas. (N. del T.)

Translation by José F. Nonidez
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A CHANGE IN THE BAR GENE OF DROSOPHILA MELANOGASTER INVOLVING FURTHER DECREASE IN FACET NUMBER AND INCREASE IN DOMINANCE[1]

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NINE FIGURES

CONTENTS

INTRODUCTION

In connection with a study of the germinal and environmental factors affecting eye-facet number in Drosophila melanogaster, which is in progress in the Zoological Laboratory at the University of Illinois, several interesting germinal changes have been observed. Some of these involve accessory factors while others are changes in the bar gene itself. Among the latter is the reverse mutation to full eye first described by H. G. May ('17) and since then observed several times by the writer. The change to be described is equally interesting. It appeared in the second low selection for facet number in white bar and involves further decrease in facet number. Like reverse mutation, it is due to a change in the bar gene itself, and not to the appearance of an accessory factor; it is in the same direction as the original bar

[1] Contributions from the Zoological Laboratory of the University of Illinois, no. 149.

mutation of full eye; it is in the same direction as the selection which was being carried on in the material, and, lastly, it has a greatly increased dominance, so that it is dominant not only over full eye, but also over bar eye.

TEMPERATURE CONTROL

Eye-facet number in Drosophila has exceptional advantages in a quantitative study of both germinal and environmental factors. The earlier studies of the germinal factors affecting this character by Zeleny and Mattoon ('15) and H. G. May ('17) were made difficult and unsatisfactory in many ways because of the disturbing presence of uncontrolled environmental factors, and it was realized that the latter must be eliminated in considerable degree before further progress could be made with the former. Experiments in this laboratory by Seyster ('19) and Krafka ('20) have shown that temperature is the most important of these factors. It affects facet number in a marked and definite manner. Adequate facilities for temperature control in the Vivarium Building have made possible the analysis of the germinal factors preexisting in the bar stocks and also the isolation of new factors as they arise.

All of the facet numbers recorded in the present paper were from individuals raised at 27° ± 0.5°. The incubator used was no. 2, made by the Chicago Surgical and Electrical Company. The heat unit is a high-resistance wire coil and contact is accomplished by the bending of a diaphragm. The platinum contact points must be kept clean. A test of the incubator as purchased showed that there was a difference of three or more degrees in the different parts of the chamber. A 12-inch General Electric desk fan with lengthened shaft so that it could be installed with the motor outside and the blades inside of the chamber has acted in a satisfactory manner in keeping the air stirred. Frequent checks were made of the temperature of the banana food in which the larvae were developing, which showed that under these conditions the food temperature differed from the air temperature by two-tenths of a degree at most. A Tycos, ribbon type, bi-record thermometer made by the Taylor Instrument Company

of Rochester, New York, was used for a continuous record of the temperature. This shows, for the period covered by the present experiments, a departure from 27° not exceeding 0.5°. The accessory tests make it probable that this applies to the temperature of the larvae as well as to that of the thermometer bulb.

The satisfactory character of the incubator was in part due to its being placed in a room with both refrigeration and heat control and maintained at 24° with an extreme departure of 1°. This constant-temperature room is cooled by air blown over brine coils and warmed by steam coils. Both systems are under automatic control installed by the Johnson Service Company of Milwaukee.

In choosing a suitable constant temperature for the experiments with germinal factors affecting facet number in the bar stocks, the primary consideration was based upon the fact that facet number decreases very strikingly with increase in temperature, and facility and accuracy in counting are of course easier the smaller the number of facets. An added inducement for using a warm temperature is the shorter developmental period. The choice of the exact temperature was more difficult. It was desired to go as high as possible without noticeable disturbance of the viability and without subjecting the work to the criticism that any germinal changes observed are the results of violent reactions to unfavorable conditions. Twenty-seven degrees centigrade was chosen after a preliminary trial had shown that at 29° viability is considerably lowered.

Flies raised in dry food frequently are much smaller than those raised in moist food, but preliminary counts under these conditions show no consistent difference in eye-facet number between the two. In figure 1 it is noticed that the magnification in each of the five cases is the same and that the size of individual facets is in general larger the larger the individual fly. In individuals of uniform size, however, as found in stocks raised under optimum conditions, the number of facets is proportional to the area of the eye as shown by Seyster ('19).

In striving for the ideal of basic stocks which will give perfectly constant facet numbers and in which any departure, no matter

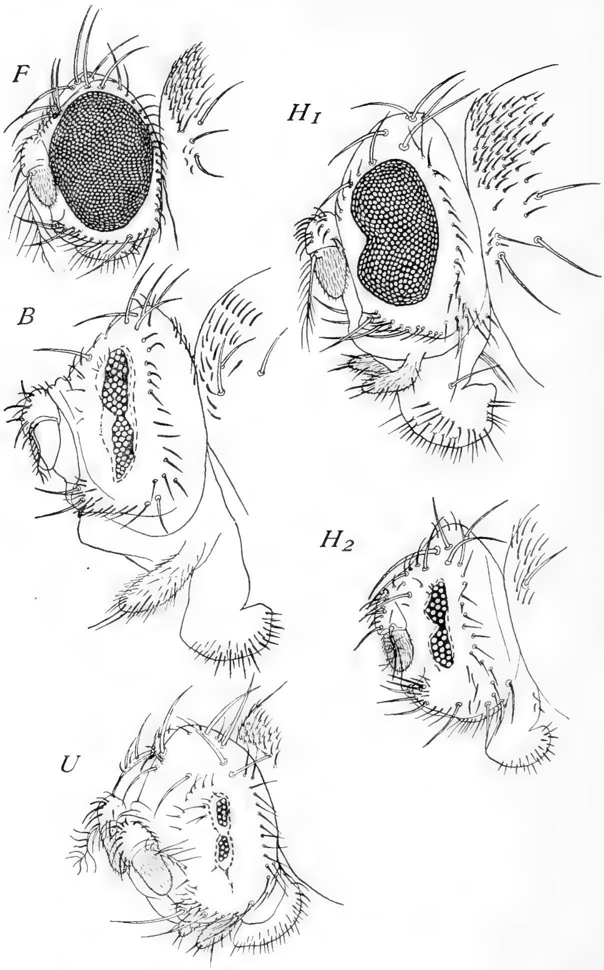

Fig. 1 Full-eye, bar, ultra-bar, and their heterozygotes. *F*, full-eye wild females. *B*, white-bar female of the second generation of low selection from which ultra-bar was derived by mutation (culture no. 144.2). *U*, ultra-bar female (no. 158.1). H_1, heterozygous female from full × bar. (no. 87.11). H_2, heterozygous female from full × ultra-bar (no. 259.3). × 60.

how small can be definitely ascribed either to a particular germinal or a particular environmental change, only a start has been made. However, analysis of the one group of factors facilitates the investigation of the other. All the material described in the present paper is preserved in alcohol as far as possible and is accessible for future reference. This explains the use of culture numbers in the descriptions.

ORIGIN, BEHAVIOR AND LOCATION OF BAR EYE

As described by Tice ('14), bar-eye appeared in a single male in a stock with full eyes. In one of my wild stocks, no. 345, full

TABLE 1

Comparison of full eye, bar, and ultra-bar, at 27°C. Females. The class distributions in the same populations are given in table 3 and figure 2

	FULL EYE[1]	BAR SECOND LOW[2]	ULTRA-BAR
Catalog number	345	144, 145	158–499 772–799
Date	1918 X 29	1917 X 9–26	1917 XII 14– 1918 VII 23
Number of individuals	10	179	1590
Mean in factorial units[3]	+26.67 ±0.24	+0.74 ±0.11	−9.79 ±0.03
Standard deviation (= coefficient of variability) in factorial units[4]	1.11 ±0.17	2.25 ±0.08	1.62 ±0.02
Lowest individual in factorial units	+24.22	−5.60	−15.93
Highest individual in factorial units	+28.02	+7.61	−4.60
Mean of facets	810.6	61.8±0.7	21.96±0.06
Lowest facet number	632	32	11
Highest facet number	924	119	35

[1] With the small number of cases represented under full eye, the standard deviation and probable error determinations of course have no great significance. The values given are those obtained by following the usual methods employed when larger numbers of cases are available. They may, however, indicate in a very general way the probable variability of the stock as compared with the others.

[2] Second low selected generation including all matings.

[3] A factorial unit is one that produces a 10 per cent change in facet number.

[4] According to the method used, standard deviation is directly a measure of variability and may be used as a coefficient of factorial variability.

eye has an average of 810.6 facets in the females and 849.8 facets in the males (tables 1 to 4). The unselected red-bar stock, as obtained by Zeleny and Mattoon ('15) for their selection work, has an average of 65.1 facets for the females and 98 for the males. The unselected white-bar stock (nos. 144 and 145) which served as the starting point of the selection line in which ultra-bar origi-

TABLE 2

Comparison of full eye, bar, and ultra-bar at 27°C. Males. The class distributions in the same population are given in table 4 and figure 3

	FULL EYE[1]	BAR SECOND LOW[2]	ULTRA-BAR
Catalog number	345	144, 145	158–499 772–799
Date	1918 X 29	1917 X 9–26	1917 XII 14–1918 VII 23
Number of individuals	10	157	1594
Mean in factorial units	+21.15 ±0.23	−3.44 ±0.14	−14.96 ±0.03
Standard deviation in factorial units	1.12 ±0.17	2.54 ±0.09	1.51 ±0.02
Lowest individual in factorial units	+19.12	−9.17	−21.05
Highest individual in factorial units	+22.49	+2.63	−9.42
Mean of facets	849.8	75.6 ±1.0	23.04 ±0.06
Lowest facet number	700	41	12
Highest facet number	980	134	40

[1] With the small number of cases represented under full eye, the standard deviation and probable error determinations of course have no great significance. The values given are those obtained by following the usual methods employed when larger numbers of cases are available. They may, however, indicate in a very general way the probable variability of the stock as compared with the others.

[2] Second low selected generation including all matings and excluding the 19-facet male from which the ultra bar stock was derived.

nated had an average of 58.8 facets for the females and 111.4 for the males. In the white bar this represents a change of −26.67 factorial units in the females and −21.15 in the males, according to the system of tabulation used in the present paper and described briefly on page 309 and more fully in a separate paper which is in preparation. A factorial unit is one that produces a 10 per cent change in facet number.

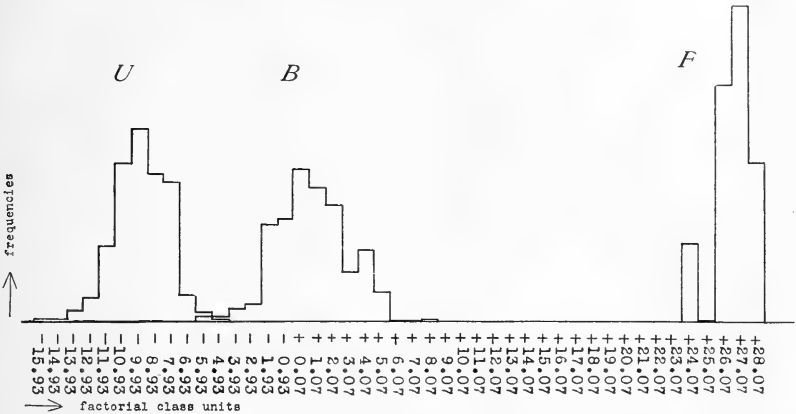

Fig. 2 The class distributions according to factorial units affecting facet numbers in populations of ultra-bar females (U), bar females of the second low selected white-eyed generation from which ultra-bar was derived (B), and full-eye (F). The class frequencies are in per cents of the whole population and are therefore directly comparable. The zero of the factorial scale is the mean value of the unselected white bar. The same material is represented in tables 1 and 3.

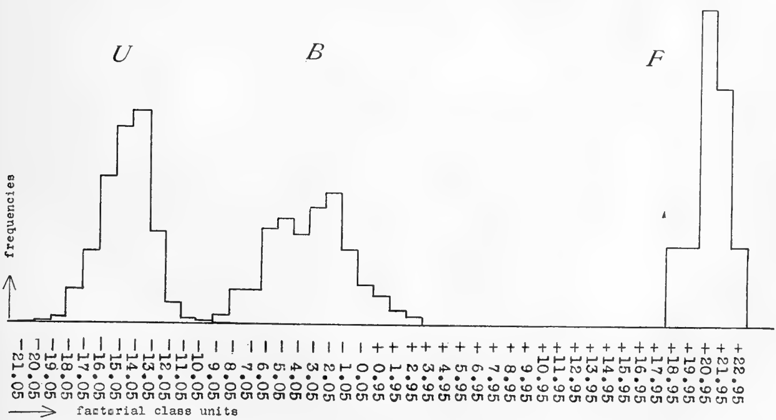

Fig. 3 The class distributions for males. The same material is represented in tables 2 and 4.

Tice further located the gene for bar eye in the sex chromosome, and on the basis of percentage of crossing over with other sex-linked factors it was put at 57 cross-over units from yellow. She considered bar as a dominant to full eye, and it is so described in various papers by Morgan and others. Strictly speaking, the heterozygous condition is intermediate between bar and full. According to the method used here, the heterozygotes between low bar (F 24) and full are +23.89 factorial units from bar and only −7.23 such units from full (table 7). If 100 per cent represents a condition of complete dominance and 0 per cent of complete recessiveness, bar has a dominance coefficient of 23.2 per cent and full of 76.8 per cent. If either is to be considered as a dominant, the term should be applied to full eye rather than to bar eye.

Bar eye has a high degree of stability, though there is an occasional mutation such as the reverse mutation to full. Such reverse mutations are apparently actual returns of the gene to the original condition. The writer has noticed several of them in his stocks and several other germinal changes, among which is the one described in the present paper.

THE ORIGIN OF ULTRA-BAR

Ultra-bar also appeared in a single male. This male with but 19 facets appeared in the second low selected generation of the white-bar line on October 20, 1917. A cross with a 44-facet sister gave a pure stock in F_3. The ultra-bar thus established has remained stable in character except for the mutations described on page 304 ff.

The average facet number is 21.96 in the females and 23.04 in the males as opposed to 61.8 and 75.6 in the second low generation of white bar from which it was derived and 810.6 and 849.8 in the full eye. In factorial units the second low generation of the white-bar line is −25.93 and −24.59 units from full. Ultra-bar is −10.53 and −11.52 units from the second low generation of white bar and −36.46 and −36.11 units from full eye. Typical representatives of full eye, bar, and ultra-bar are shown in figure 1. Table 1 gives the values of the means, the ranges, and

the standard deviations. In the case of factorial units the standard deviation may be used directly as the coefficient of variation. The values for full eye are based upon such a small number of cases wholly because of the tedious character of the count. In the bar stocks counts can be made by placing the flies on their sides on a small block of wood with a surface painted black and so inclined as to bring the eye facets in a horizontal plane under the microscope. The rows of facets can then be followed by the observer and counts can be made with a fair degree of accuracy. In practice there is, however, always a small per cent of error which increases with the facet number. In full eye the large number of facets combined with the curvature of the eye makes this procedure impossible. It is necessary to resort to the mounting of the eye on a slide under a cover-glass. The non-chitinous parts are removed with caustic potash and the faceted part mounted in Canada balsam and flattened out under a cover-glass. With a high objective, a mechanical stage, and cross hairs in the ocular, it is possible to count the facets with a fair degree of accuracy, but it is not profitable to count a large number of individual eyes.

Tables 3 and 4 and figures 2 and 3 give comparisons of the range and variability of the three stocks in graphic form. There can be no question that the stocks are absolutely distinct. Bar and ultra-bar barely overlap in the females and not at all in the males. As shown in the tables, the variability of ultra-bar, here expressed directly by the standard deviation, is significantly less than that of the bar stock from which it was derived, and this difference between bar and ultra-bar remains throughout the selection generations. Figures 2 and 3 bring out the same point. The standard deviation value for full eye of course has no special significance because of the small number of cases. It may perhaps indicate that full eye is less variable than bar.

TABLE 3

Variability of full, bar, and ultra-bar. Females

CLASSES IN FACTORIAL UNITS	CLASSES IN FACETS	FULL EYE		BAR SECOND LOW SELECTED GENERATION		ULTRA-BAR	
		Number	Per cent	Number	Per cent	Number	Per cent
−15.93	11					3	0.2
−14.93	12					3	0.2
−13.93	13+					2½	1.4
−12.93	15+					49½	3.1
−11.93	16–17					155	9.7
−10.93	18–19					317	19.9
− 9.93	20–21					387	24.3
− 8.93	22–23					294	18.4
− 7.93	24–26					282	17.7
− 6.93	27–29					57	3.5
− 5.93	30–32			1	0.6	17	1.1
− 4.93	33–35			1	0.6	4	0.3
− 3.93	36–39			3	1.7		
− 2.93	40–43			4	2.2		
− 1.93	44–48			22	12.3		
− 0.93	49–53			23	12.8		
+ 0.07	54–59			34	19.0		
+ 1.07	60–65			30	16.8		
+ 2.07	66–72			26	14.5		
+ 3.07	73–80			11	6.1		
+ 4.07	81–88			16	9.0		
+ 5.07	89–97			7	3.9		
+ 6.07	98–107						
+ 7.07	108–118						
+ 8.07	119–131			1	0.6		
+ 9.07	132–145						
+10.07	146–160						
+11.07	161–177						
+12.07	178–196						
+13.07	197–217						
+14.07	218–240						
+15.07	241–265						
+16.07	266–293						
+17.07	294–324						
+18.07	325–358						
+19.07	359–396						
+20.07	397–438						
+21.07	439–484						
+22.07	485–535						
+23.07	536–591						
+24.07	592–653	1	10				
+25.07	654–722						
+26.07	723–798	3	30				
+27.07	799–882	4	40				
+28.07	883–975	2	20				
Totals		10	100	179	100	1590	100

TABLE 4

Variability of full, bar, and ultra-bar. Males.

CLASSES IN FACTORIAL UNITS	CLASSES IN FACETS	FULL EYE		BAR SECOND LOW SELECTED GENERATION		ULTRA-BAR	
		Number	Per cent	Number	Per cent	Number	Per cent
−21.05	12					1	0.1
−20.05	13+					2½	0.2
−19.05	15+					12½	0.8
−18.05	16–17					68	4.3
−17.05	18–19			1[1]	0.6	148	9.3
−16.05	20–21					294	18.4
−15.05	22–23					395	24.8
−14.05	24–26					429	26.9
−13.05	27–29					185	11.6
−12.05	30–32					43	2.7
−11.05	33–35					11	0.7
−10.05	36–39					4	0.3
− 9.05	40–43			2	1.3	1	0.1
− 8.05	44–48			7	4.4		
− 7.05	49–53			7	4.4		
− 6.05	54–59			19	12.0		
− 5.05	60–65			21	13.3		
− 4.05	66–72			18	11.4		
− 3.05	73–80			23	14.6		
− 2.05	81–88			26	16.5		
− 1.05	89–97			15	9.5		
− 0.05	98–107			8	5.1		
+ 0.95	108–118			6	3.8		
+ 1.95	119–131			3	1.9		
+ 2.95	132–145			2	1.3		
+ 3.95	146–160						
+ 4.95	161–177						
+ 5.95	178–196						
+ 6.95	197–217						
+ 7.95	218–240						
+ 8.95	241–265						
+ 9.95	266–293						
+10.95	294–324						
+11.95	325–358						
+12.95	359–396						
+13.95	397–438						
+14.95	439–484						
+15.95	485–535						
+16.95	536–591						
+17.95	592–653						
+18.95	654–722	1	10				
+19.95	723–798	1	10				
+20.95	799–882	4	40				
+21.95	883–975	3	30				
+22.95	976–1078	1	10				
Totals		10	100	158	100	1594	100

[1] The mutant from which the ultra-bar stock arose.

CONSTANCY OF ULTRA-BAR

The single mutant male appeared on October 20, 1917, and the new stock was isolated in pure condition on December 27th. Numerous counts at 27° show no essential change in character except for the mutants mentioned in a later paragraph. The mean, range, and standard deviation remain essentially unchanged. The data are given in tables 5 and 6. During the period of establishment of the stock it was subjected to selection, but without any noticeable effect. There is no indication that this selection had any part in the production of the constancy. The selection data are treated in a separate paper. Later counts made during July, 1919, and not given in the tables show that the stock had remained without essential change for twenty months. The differences in the various cultures as shown in tables 5 and 6 are in some cases well within the probable error of random sampling, but in several cases the departures can not be explained in this way. For instance, take such a difference as that between the means of cultures no. 178 and no. 184 which is equal to 1.86 units. Its probable error, $E_D = \pm\sqrt{E_1^2 + E_2^2} = \pm 0.17$, is only one-eleventh of the difference. Correspondingly, the difference in the males of the two cultures is 1.71 units and the probable error is 0.16. These differences are therefore in all probability not due to random sampling. Their lack of permanence indicates that they are environmental and not germinal.

On four occasions individuals have appeared in the ultra-bar stock which differ markedly from the ordinary ones. Three of these may be considered as probably reverse mutations to full and the fourth as a new mutation upward in the direction of bar.

Mutant A. On July 9, 1918, there appeared in bottle no. 496.4 of the ultra-bar stock at 27°C. a female with 46 facets in the left eye and 50 in the right eye. The upper limit of ultra-bar stock females is 35 facets (table 5). It appeared that this female might be a heterozygote with one ultra-bar and one full-eye factor as the facet range of 54 such heterozygotes in test no. 357, table 7, is 28 to 49. Accordingly, she was mated with four

of her brothers and gave according to expectation both full-eye and ultra-bar sons and ultra-bar and heterozygous daughters. Other crosses followed expectation in a similar manner.

That she is not the result of contamination is indicated, first, by the fact that she is white-eyed, and that hypothesis therefore necessitates contamination by a white full-eyed fly. Trap tests showed that there were no such flies free in the laboratory, though other kinds were caught. In the second place, only a single such individual appeared. Thirdly, the fact that she is a heterozygote makes it improbable that she is a stranger in the bottle. Fourthly, the general laboratory procedure is the same as that pursued when the reverse mutations of bar appeared, and the arguments given for those cases by May (1917) hold in this case also.

Deficiency tests were made. If a piece of the chromosome carrying the ultra-bar factor has dropped out, either the forked gene which is −0.5 units from ultra-bar or the fused gene which is +2.5 units away or both would in all probability be carried with it. When full males derived from a cross of the mutant female with ultra-bar males are mated with heterozygous fused females, they should give fused sons if there is deficiency on the plus side of ultra-bar. Such crosses gave no fused or forked sons and deficiency cannot be considered as the explanation of the mutant.

Mutant B. In a cross between wild red-eyed females and a 22-facet white male from bottle no. 150 sp., 59 of the females were heterozygotes according to expectation and there was one full-eyed female. This exceptional female was crossed with wild red males and gave 23 full-eyed females and 25 full-eyed males. Both chromosomes therefore have the full-eyed factor, and the case is not due to a failure of the ultra-bar factor to dominate. Dr. A. H. Sturtevant has suggested that this may be a case of non-disjunction. Unfortunately the eye color was not recorded at the crucial point.

Mutant C. A single full-eyed white male appeared in the white ultra-bar stock no. 158.1 on December 26, 1917. It was shown by test no. 291.1 to be like ordinary full-eye, but no further tests were made.

TABLE 5

Constancy of ultra-bar females. 27°.0±0.5

CATALOG NUMBERS	DATES	NUMBER OF INDIVIDUALS	FACTORIAL UNITS						FACETS		
			Mean		Standard deviation		Lowest	Highest	Mean	Lowest	Highest
158	1917 XII 14–I 2	76	−9.44	±0.14	1.79	±0.10	−14.10	−4.60	21.86	13	35
164	1918 I 4–I 23	186	−9.76	±0.08	1.64	±0.06	−14.93	−5.60	21.04	12	32
170	1918 I 15–II 2	83	−9.77	±0.10	1.36	±0.07	−13.43	−7.26	20.87	14	27
178	1918 I 16–II 14	82	−8.58	±0.10	1.31	±0.07	−11.18	−5.93	23.68	18	31
184	1918 II 6–III 1	79	−10.44	±0.14	1.85	±0.10	−15.93	−5.26	19.77	11	33
190	1918 II 16–III 11	90	−10.20	±0.11	1.62	±0.08	−14.10	−6.60	20.10	13	29
196	1918 II 22–III 25	96	−9.75	±0.10	1.47	±0.07	−14.10	−6.93	20.98	13	28
216	1918 III 26–IV 26	100	−9.68	±0.10	1.43	±0.07	−13.43	−6.26	21.22	14	30
226	1918 IV 26–V 14	55	−9.51	±0.13	1.46	±0.09	−12.76	−6.60	21.62	15	29
232	1918 V 11–V 29	85	−10.46	±0.09	1.23	±0.06	−14.10	−7.93	19.52	13	25
238	1918 V 21–VI 4	33	−9.99	±0.15	1.26	±0.10	−12.18	−7.60	20.30	16	26
371	1918 VI 18–VII 1	50	−9.88	±0.16	1.72	±0.12	−15.93	−7.93	20.54	11	25
496–499	1918 VII 2–VII 23	575	−9.80	±0.05	1.64	±0.03	−15.93	−5.93	20.91	11	31[1]
800 a–d	1917 XII 14–1918 VII 23	1590	−9.79	±0.03	1.62	±0.02	−15.93	−4.60	20.96±0.06	11	35

[1] Excluding one 46-facet female, which was shown by test to be heterozygous ultra-bar with full (catalog nos. 504 and 505).

TABLE 6

Constancy of ultra-bar males. 27°.0±0.5

CATALOG NUMBERS	DATES	NUMBER OF INDIVIDUALS	FACTORIAL UNITS				FACETS		
			Mean	Standard deviation	Lowest	Highest	Mean	Lowest	Highest
145.1	1917 X 20	—	−16.80[1]				19.00		
158	1917 XII 14–I 2	73	−15.13 ±0.12	1.57 ±0.09	−17.80	−12.05	22.77	17	31
164	1918 I 4–23	168	−15.09 ±0.09	1.78 ±0.07	−18.88	−9.67	22.86	15	39
170	1918 I 15–II 2	83	−14.99 ±0.09	1.20 ±0.06	−18.88	−12.38	22.80	15	30
178	1918 I 16–II 14	93	−14.01 ±0.10	1.48 ±0.07	−16.80	−9.42	25.42	19	40
184	1918 II 6–III 1	72	−15.72 ±0.13	1.67 ±0.09	−19.55	−11.38	21.26	14	33
190	1918 II 16–III 11	93	−15.46 ±0.09	1.30 ±0.06	−19.55	−12.72	21.81	14	29
196	1918 II 22–III 25	103	−14.94 ±0.09	1.33 ±0.06	−18.30	−10.42	22.87	16	36
216	1918 III 26–IV 26	98	−14.98 ±0.10	1.42 ±0.07	−18.88	−11.38	23.22	15	33
226	1918 IV 26–V 14	61	−14.53 ±0.12	1.37 ±0.08	−18.88	12.38	24.11	15	30
232	1918 V II–V 29	76	−15.41 ±0.10	1.25 ±0.07	−18.30	−12.05	21.93	16	31
238	1918 V 21–VI 14	31	−15.11 ±0.17	1.39 ±0.12	−18.30	−13.05	22.52	16	28
371	1918 VI 18–VII 1	43	−14.77 ±0.12	1.13 ±0.08	−18.30	−12.72	23.37	16	29
476–499	1918 VII 2–VII 23	600	−14.90 ±0.04	1.50 ±0.03	−21.05	−10.72	23.22	12	35
800 a–d	1917 XII 14–1918 VII 23	1594	−14.96 ±0.03	1.51 ±0.02	−21.05	−9.42	23.04±0.06	12	40[2]

[1] The original mutant.

[2] Excluding one full eye.

Mutant D. In bottle no. 196.2 of the ultra-bar stock there appeared on March 20, 1918, both males and females with a facet number coming within the range of bar of the low selection line. When this particular mass culture was started on February 2, 1918, all the individuals were ultra-bar. The number of individuals with the new character makes it probable that the mutation occurred at least two generations before the first observation was made. A 62-facet and two 99-facet males were mated separately with full-eyed wild females and gave heterozygotes which differed from those of ultra-bar × full and also of bar × full. The mean value in about five hundred flies as determined by an estimate without counts is very distinctly between the other two types of heterozygotes, and the range is extended so as to overlap their ranges. This extension of the range indicates the probable presence of accessory factors, but its limits make it seem that the new form is a mutation of ultra-bar in the direction of bar which is not exactly like bar.

Other cases that may be considered as mutations are given in the section on the determination of the locus of ultra-bar (p. 311).

The frequency of occurrence of mutations in ultra-bar is about the same as that in bar, and the stability of the two stocks may be considered to be of about the same order.

THE CHANGE IN DOMINANCE

Even more striking than the difference in facet number between bar and ultra-bar is the difference in dominance. A heterozygote between ultra-bar and full comes very close to ultra-bar, while the heterozygote between bar and full is closer to full than to bar (fig. 1 and table 7). For purposes of comparison, the following method of determining the coefficient of dominance has been devised. Let A and A′ be the two members of an allelomorphic pair of factors, then the coefficient of dominance $C_D = \dfrac{AA - AA'}{AA - A'A'} \times 100$ in which AA and A′A′ are the mean values, respectively, of the homozygous stocks in factorial units and AA′ is the mean value of the heterozygous individuals.

As discussed in a separate paper, the value of a factor or combination of factors affecting facet number is put on the basis of 10 per cent units. An arbitrary point, the mean facet value in the unselected stock, is taken as the point of reference, and distribution classes are so arranged that the facet range of each class is 10 per cent of the mean facet value of that class. In this scheme any facet value may be represented as being a departure plus or minus a certain number of 10 per cent units from the point of reference. The method is based on the view that

TABLE 7

Dominance values in ultra-bar, bar, and full eye

FEMALES	CATALOG NUMBERS	NUMBERS OF INDIVIDUALS	MEAN FACET VALUES	MEANS IN FACTORIAL UNITS	FACTORIAL UNITS FROM HETEROZYGOTES	DOMINANCE IN PER CENTS
Ultra-bar stock	158–499	1590	21.96	−9.79	−5.53	84.8
Full stock	345	10	810.6	+26.67	+30.93	15.2
Heterozygotes	357	54	36.54	−4.26		
Low selected bar (F_{24})	391.2	129	35.1	−4.45	+23.89	23.2
Full stock	345	10	810.6	+26.67	−7.23	76.8
Heterozygotes	769	19	399.9	+19.44		
Ultra-bar stock	158–499	1590	21.96	−9.79	−1.84	82.6
Low selected bar (F_2)	144–145	179	61.8	+0.74	+8.69	17.4
Heterozygotes	742	87	25.7	−7.95		

change in facet number is not a matter of accretion, but that the whole facet-producing mass is involved. According to this view, the factorial value of the difference between a 50-facet and a 55-facet individual is the same as that of the difference between a 500- and a 550-facet individual. In other words, an environmental or germinal change in what would otherwise have been a 500-facet stock and which produces instead 550 facets would, if acting upon a 50-facet stock, produce 55 facets and not 100 facets. The ordinary tabulations of variation in which classes have equal character values over the whole range of variation do not give equal factorial values.

Using factorial values and reducing facet numbers to departures plus or minus from the mean value of the unselected white-bar stock, the following percentage coefficients of dominance were determined:

a. Low selected bar (F_{24}) over full eye

$$= \frac{\text{full} - \text{heterozygote}}{\text{full} - \text{low selected bar } (F_{24})} = \frac{26.67 - 19.44}{26.67 - (-4.45)} \times 100$$

$= 23.2$ per cent.

b. Full eye over low selected bar (F_{24})

$$= \frac{\text{low selected bar } (F_{24}) - \text{heterozygote}}{\text{low selected bar} \qquad - \text{full}}$$

$$= \frac{-4.45 - 19.44}{-4.45 - 26.67} \times 100 = 76.8 \text{ per cent.}$$

c. Ultra bar over full eye $= \dfrac{\text{full} - \text{heterozygote}}{\text{full} - \text{ultra-bar}}$

$$= \frac{+26.67 - (-4.26)}{+26.67 - (-9.79)} \times 100 = 84.6 \text{ per cent.}$$

d. Full eye over ultra-bar $= \dfrac{\text{ultra-bar} - \text{heterozygote}}{\text{ultra-bar} - \text{full}}$

$$= \frac{-9.79 - (-4.26)}{-9.79 - 26.67} \times 100 = 15.4 \text{ per cent.}$$

e. Ultra-bar over low selected bar (F_2)

$$= \frac{\text{low selected bar } (F_2) - \text{heterozygote}}{\text{low selected bar} \qquad - \text{ultra-bar}}$$

$$= \frac{+0.74 - (-7.95)}{+0.74 - (-9.79)} \times 100 = 82.6 \text{ per cent.}$$

f. Low selected bar (F_2) over ultra-bar

$$= \frac{\text{ultra-bar} - \text{heterozygote}}{\text{ultra-bar} - \text{low selected bar } (F_2)}$$

$$= \frac{-9.79 - (-7.95)}{-9.79 - 0.74} \times 100 = 17.4 \text{ per cent.}$$

With three genes at the same locus all affecting facet number, it is possible to make the interesting comparisons whose quantitative values have just been determined. Obviously, dominance has been strikingly increased by the change which transformed bar into ultra-bar. Bar pulls full down only 23.2 per

cent of the distance between the two, while ultra-bar pulls full down 84.6 per cent. Correspondingly, ultra-bar pulls bar 82.6 per cent of the distance between them. The difference in dominance is shown also in the tabulations of the facet values of the daughters of a cross between a heterozygous female and a bar male as compared with the daughters of a heterozygous female with an ultra-bar male (tables 7 and 8 and figs. 6 and 8). In the first case (fig. 6) the distribution of values is distinctly bimodal, because the mean values of heterozygous and bar females are far apart. In the second class the distribution is essentially unimodal, because the values of heterozygous and ultra-bar females are close together.

THE LOCATION OF THE ULTRA-BAR GENE

That the new factor is located in the sex chromosome is indicated by the results of numerous matings as given in tables 8 and 9. Bar females mated with ultra-bar males give intermediate females and bar males (figs. 4 and 5). Heterozygous females mated with bar males give bar and intermediate females and bar and ultra-bar males (figs. 6 and 7). Heterozygous females mated with ultra-bar males give bar and ultra-bar males (fig. 9) and presumably ultra-bar and heterozygous females, though the two populations are so close together that the overlapping gives an approach to a normal variation curve (fig. 8). The compound character of the curve is indicated by its greater variability as compared with either ultra-bar alone or heterozygotes alone, as shown in table 8.

If ultra-bar is the result of an accessory factor acting in conjunction with an unchanged bar gene, it should be possible by crossing over to separate the two and thus obtain the original bar.

Ultra-bar females crossed with full males give in F_1 $\dfrac{B'U}{\underline{\text{—}\times\text{—}}}$ females and $\dfrac{B'U}{\text{—}\times\text{—}}$ males. If there is no crossing over F_2 should give $\dfrac{B'U}{\text{—}\times\text{—}}$ or ultra-bar males, ——— or full males, $\begin{array}{c}B'U\\ \text{—}\times\text{—}\\ \text{—}\times\text{—}\\ B'U\end{array}$

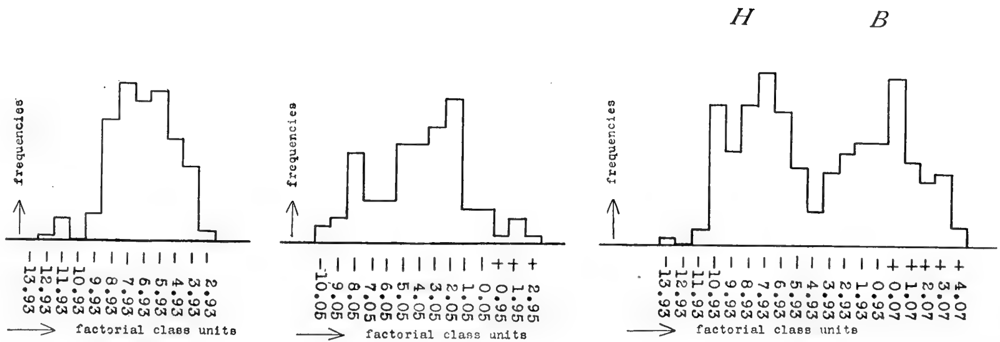

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Fig. 4 The class distributions according to factorial units for heterozygous females from bar females by ultra-bar males. The same material is given in table 8.

Fig. 5 Bar males from bar females by ultra-bar males. The same material is given in table 9.

Fig. 6 Heterozygous and bar females from heterozygous females by bar males. The same material is given in table 8.

or ultra bar females and $\dfrac{\overset{B'U}{-\times-}}{\rule{3em}{0.4pt}}$ or heterozygous females with ultra-bar and full-eye factors. If crossing over occurs there should be in addition to the above $\overset{B'}{-\times-}$ or bar males, $\overset{U}{-\times-}$ or full males with an U accessory factor, $\begin{array}{c} B' \\ -\times\rule{2.5em}{0.4pt} \\ -\times-\times- \\ B' \;\; U \end{array}$ or heterozygous females with bar and ultra-bar factors and $\begin{array}{c} U \\ \rule{2.5em}{0.4pt}\times- \\ -\times-\times- \\ B' \;\; U \end{array}$ or heterozygous females with ultra-bar and full factors plus an U accessory factor.

As bar males are readily distinguishable from ultra-bars, the test of the hypothesis is the presence or absence of bar males in F_2. The other differences indicated by the formulae can probably not be detected with certainty as the effect of an accessory U factor upon full is unknown, the heterozygote between bar and ultra-bar is so close to pure ultra-bar that it cannot be distinguished with certainty and the effect of an extra accessory U upon the heterozygote between ultra-bar and full is unknown.

Full females crossed with ultra-bar males give in F_1 heterozygous females and full males. The expected F_2 males are the same as those in the reciprocal cross. If there is no crossing over, the expected females are $\dfrac{\overset{B'U}{-\times-}}{\rule{3em}{0.4pt}}$ or heterozygotes with low facet numbers and $\dfrac{\rule{3em}{0.4pt}}{\rule{3em}{0.4pt}}$ or full females. If crossing over occurs there are to be expected in addition to the above $\dfrac{\overset{B'}{-\times-}}{\rule{3em}{0.4pt}}$ or heterozygotes with high facet number and $\dfrac{\overset{U}{-\times-}}{\rule{3em}{0.4pt}}$ or full females with a U accessory factor.

The heterozygotes between ultra-bar and full are so much lower than those between bar and full that the two can be easily differentiated. The facet values of the other differences are unknown.

TABLE 8

Females from matings involving bar and ultra-bar

CHARACTER OF MATING		STOCK	STOCK	BAR ♀ × ULTRA-BAR ♂	HETEROZYGOUS ♀ × ULTRA-BAR ♂		HETEROZYGOUS ♀ × ULTRA-BAR ♂
		Figure 2	Figure 2	Figure 4	Figure 6	Figure 6	Figure 8
Catalog numbers		144, 145	772–799	737, 742	740		738, 744, 746, 748
Offspring		Bar	Ultra-bar	Hererozygotes	Heterozygotes	Bar	Heterozygotes and ultra-bar
Factorial classes	Facet value						
−15.93	11		0.2				
−14.93	12		0.2				1.4
−13.93	13+		1.4	0.6	1.0		1.4
−12.93	15+		3.1	2.9			1.4
−11.93	16–17		9.7		2.0		8.2
−10.93	18–19		19.9	3.4	17.6		21.8
− 9.93	20–21		24.3*	14.9	11.8		30.0
− 8.93	22–23		18.4	19.5	17.6*		24.4
− 7.93	24–26		17.7	17.2*	21.6		50.4*
− 6.93	27–29		3.5	18.4	16.7		28.6
− 5.93	30–32	0.6	1.1	12.6	9.8		13.6
− 4.93	33–35	0.6	0.3	9.2	2.0	2.3	13.6
− 3.93	36–39	1.7		1.1		9.3	4.0
− 2.93	40–43	2.2				11.6	1.4
− 1.93	44–48	12.3				12.8	
− 0.93	49–53	12.8				12.8*	
+ 0.07	54–59	19.0				20.9	
+ 1.07	60–65	16.8*				10.5	
+ 2.07	66–72	14.5				8.1	
+ 3.07	73–80	6.1				9.3	

+ 4.07	81–88	9.0				2.3	
+ 5.07	89–97	3.9					
+ 6.07	98–107						
+ 7.07	108–118						
+ 8.07	119–131	0.6					
Number of individuals		179	1590	87	102	86	147
Mean	Bar	+0.74 ±0.11				−0.51 ±0.16	
	Ultra-bar		−9.79 ±0.03				
	Heterozygotes			−7.95 ±0.14	−8.59 ±0.12		
	Ultra-bar and heterozygotes						−8.37 ±0.12
Standard deviation	Bar	2.25 ±0.08				2.25 ±0.11	
	Ultra-bar		1.62 ±0.02				
	Heterozygotes			1.93 ±0.10	1.79 ±0.08		
	Ultra-bar and heterozygotes						2.11 ±0.08

* Class containing the mean value.

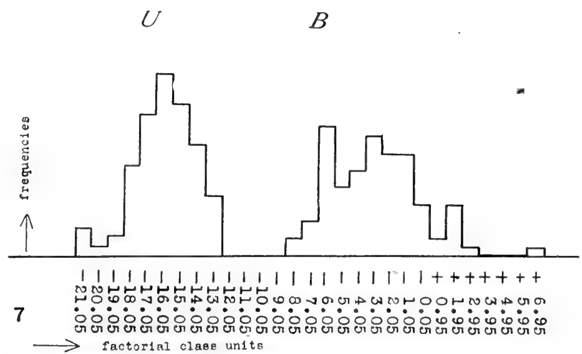
TABLE 9

Males from matings involving bar and ultra-bar

CHARACTER OF MATING		STOCK	STOCK	BAR ♀ × ULTRA-BAR ♂	HETEROZYGOUS ♀ × BAR ♂		HETEROZYGOUS ♀ × ULTRA-BAR ♂	
		Figure 3	Figure 3	Figure 5	Figure 7		Figure 9	
Catalog numbers		144, 145	772–799	738, 743	741		739, 745, 747, 749	
Offspring		Bar	Ultra-bar	Bar	Bar	Ultra-bar	Bar	Ultra-bar
Factorial classes	Facet value							
−21.05	12		0.1			3.8		
−20.05	13+		0.2			1.3		
−19.05	15+		0.8			2.5		
−18.05	16–17		4.3			11.4		1.3
−17.05	18–19		9.3			17.7		14.3
−16.05	20–21		18.4			22.8*		19.5
−15.05	22–23		24.8*			19.0		19.5*
−14.05	24–26		26.9			13.9		31.2
−13.05	27–29		11.6			7.6		13.0
−12.05	30–32		2.7					1.3
−11.05	33–35		0.7					
−10.05	36–39		0.3	2.2				
− 9.05	40–43	1.3	0.1	3.4			2.2	
− 8.05	44–48	4.4		11.2	2.2		6.6	
− 7.05	49–53	4.4		5.6	4.3		7.7	
− 6.05	54–59	12.0		5.6	16.1		8.8	
− 5.05	60–65	13.3		12.4	8.6		12.1	
− 4.05	66–72	11.4		12.4*	10.7		7.7	
− 3.05	73–80	14.6*		14.6	15.1*		11.0*	
− 2.05	81–88	16.5		18.0	12.9		13.2	

− 1.05	89–97	9.5		4.5	12.9		9.9	
− 0.05	98–107	5.1		4.5	6.5		8.8	
+ 0.95	108–118	3.8		1.1	2.2		8.8	
+ 1.95	119–131	1.9		3.4	6.5			
+ 2.95	132–145	1.3		1.1	1.1		1.1	
+ 3.95	146–160						1.1	
+ 4.95	161–177						1.1	
+ 5.95	178–196							
+ 6.95	197–217				1.1			
Number of individuals		157	1594	89	93	79	91	77
Mean	Bar	−3.44 ±0.14		−4.05 ±0.21	−2.93 ±0.19		−3.23 ±0.21	
	Ultra-bar		−14.96 ±0.03			−16.08 ±0.14		−14.96 ±0.10
Standard deviation	Bar	2.54 ±0.09		2.89 ±0.15	2.79 ±0.13		3.06 ±0.15	
	Ultra-bar		1.51 ±0.02			1.85 ±0.10		1.35 ±0.07

* Class containing the mean value.



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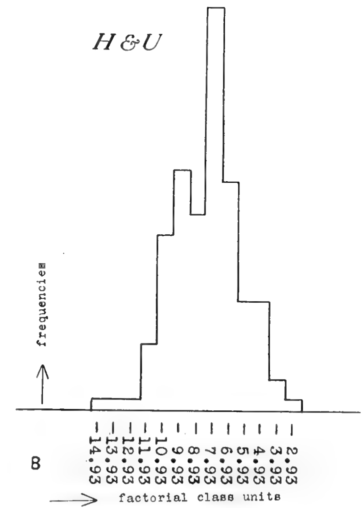

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Fig. 7 Ultra-bar and bar males from heterozygous females by bar males. The same material is given in table 9.

Fig. 8 Heterozygous and ultra-bar females from heterozygous females by ultra-bar males. The two populations are so close together as to be indistinguishable on superficial inspection. The same material is given in table 8.

The results of these comparisons are given in table 10. Males with 40 facets or less are listed as ultra-bar and those with a few facets above 40 as ultra-bar +. Those that are considerably above 40 are classed as high males and might have been considered as probably belonging to bar if no tests had been made of them. Among the females are included the heterozygotes. Those that agree in facet number with the heterozygotes of F_1, having 49 facets or less according to mating no. 357 (table 7), are listed as low heterozygotes. Those that are higher than this

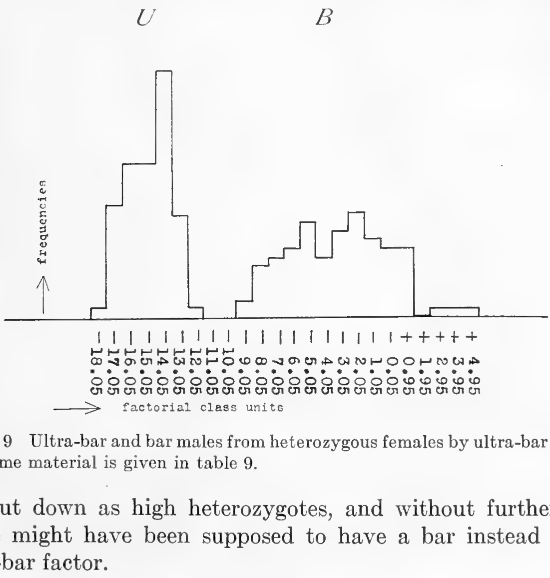

Fig. 9 Ultra-bar and bar males from heterozygous females by ultra-bar males. The same material is given in table 9.

are put down as high heterozygotes, and without further evidence might have been supposed to have a bar instead of an ultra-bar factor.

TABLE 10

F_2 from ultra-bar × full and full × ultra-bar

	ULTRA-BAR	ULTRA-BAR+	TOO HIGH FOR ULTRA-BAR	LOW HETEROZYGOTES	HIGH HETEROZYGOTES
Males	1235	3	3		
Females				553	4

Among the F_2 males of both the reciprocal crosses there are 1235 males within the range of ultra-bar, three of which come just above this range having respectively 42, 42, and 44 facets, and three which are considerably higher having 51, 60, and 88 facets. Of the first three, one of the 42-facet males was tested by crossing to full and shown by its dominance to be an ultra-bar. It is probable from this and other evidence that this slight extension of the range of ultra-bar is due to accessory factors introduced by the full stock. The other three males are of a different character. The 60-facet male died without a test. The 51- and 88-facet males when mated to full gave heterozygotes which were higher than those of ultra-bar × full, but on the other hand lower than those of bar × full. These heterozygotes agree in character with those of the mutant in the pure ultra-bar stock which was described above on p. 308. The high exceptional males cannot therefore be considered as bar males produced by the separation of the U factor from B′ by crossing over.

Among the F_2 females of the cross between full females and ultra-bar males there are 553 with the characteristics of typical low heterozygotes of the F_1 generation and four with higher facet numbers. One of the latter has 154 facets and is considerably below the range of heterozygotes between full and bar as given in table 9. The other three females come within this range. The one which was tested by crossing to full, however, gave only ultra-bar and full males instead of bar and full as its facet number seemed to indicate that it should give. It is therefore an instance of failure of the ultra-bar factor to dominate full in the ordinary manner. As in the case of the males, crossing over cannot be given as an explanation of these exceptional females, though it is unfortunate that some of them died before a test could be made. However, even if all the exceptional untested males and females were classed as bars produced by crossing over of an assumed accessory ultra-bar factor, the locus of this factor would have to be placed very close to bar. Absolute proof is impossible because it can always be held that the new gene is so close to the old one that crossing over cannot certainly be expected in any

practicable number of individuals. Besides there may be a 'no-crossover' accessory factor.

The reasonable conclusion to be drawn from the evidence is that ultra-bar has the same locus as bar or a locus so close to it that the two are a single unit in all demonstrable cases. It follows that ultra-bar has been produced by a change in the bar gene, and not by a change at some other locus.

CONCLUSIONS

Eye-facet number in Drosophila furnishes an excellent material for the quantitative study of both germinal and environmental factors. The demonstration of the striking and regular temperature effect makes possible the recognition and analysis of germinal factors. As outlined in preliminary reports (see bibliography) selection for facet number in bar-eye has shown that the effect of selection here is the result, first, of a sorting out of germinal diversities in the original stock and, second, of the isolation of new diversities as they arise. Besides occasional reverse mutations to full, the original diversities as well as the new changes have been due to accessory factors. The mutation described in the present paper, however, resembles the reverse mutations in that it involves change in the bar gene. The effect here, however, is essentially an intensification of that produced by bar. There is further decrease in facet number and a great increase in dominance.

It is interesting to note also that the change occurred in a line in which low selection was being carried on. In the light of all the evidence concerning the direction of mutations, no special significance can be attached to this fact at present. For instance, reverse mutations to full occur in the low selection lines of bar and in ultra-bar as well as in the high selection lines. While, therefore, a connection between the direction of selection and the direction of mutations in general seems improbable, the matter is of sufficient importance to warrant careful record of all instances.

The constancy of the new gene seems to be of about the same order as that of bar. Reverse mutations to full occur in both. In ultra-bar there is also a reverse mutation in the direction of bar, but apparently not to the same point because, while the mutant comes within the facet range of bar, it does not have the same dominance over full.

The locus of the new gene is the same as that of bar or is so close to it that our methods of analysis are not capable of separating the two. The crossing-over test is the only one that can be applied, and it has the obvious difficulties due to the improbability of any crossing over between two genes which are very close together, and it is also subject to the criticism that the crossing over may itself be inhibited by an accessory factor. However, it is our only test and its findings must be taken at their face value.

In the particular test applied, the presence or absence of crossing over in F_2 of crosses between ultra-bar and full, a few individuals appeared which came within the facet range of bar. The untested ones might of course have been bar. It is unfortunate that absolute certainty was not obtained because of the interest in progressive changes of the genes. Most of the progressive changes in characters observed in Drosophila are due to accessory factors and not to changes in a single gene.

The presence of three genes at the same locus made possible an attempt to see if ultra-bar bears a direct quantitative relation to bar. If this is true, it might be considered as merely two or more bar units closely held together. The values for full, full $\times$ bar, bar, ultra-bar $\times$ full, ultra-bar $\times$ bar, and ultra-bar females could in that case be represented as resulting from different amounts of a common inhibitor of facet number and there should be a consistent relation in the facet values. This, however, is not true, and it seems probable therefore that the change from bar to ultra-bar is specific and not merely a quantitative intensification of the bar factor.

SUMMARY

1. The recognition of a pronounced and regular effect of temperature upon facet number in Drosophila has made possible a more accurate analysis of the germinal changes taking place in the bar-eyed races.

2. A single male with but 19 facets appeared in the second generation of downward selection in white bar, the normal range of that generation being 41 to 134 facets.

3. From this single male there was derived by crossing with his sisters a new race which has been called ultra-bar. The symbol for its gene is B′U.

4. The mean facet value of the males of this race is 23 as compared with 75.6 in the parental stock.

5. The ranges of ultra-bar and bar do not overlap in the males and barely overlap in the females.

6. The new stock has remained unchanged for over twenty months except for the appearance of a few individuals with marked departures from the normal range.

7. Among these mutations there have appeared reversals to full-eye and returns to a condition resembling bar in facet number, but differing from it in dominance.

8. Ultra-bar differs strikingly from bar in having a much greater dominance over full, 84.8 per cent as compared with 23 per cent.

9. Correspondingly ultra-bar has a dominance of 82.6 per cent over bar.

10. The ultra-bar gene is located in the sex chromosome.

11. Crossing-over tests show that the new gene has the same locus as bar or is so close to it as to be identical for all practical purposes.

12. The change involved is to be considered as a change in the bar gene itself which is in the direction of the original change from full to bar.

13. The dominance relations, however, make it improbable that ultra-bar can be considered merely as a quantitative increase in the bar reaction.

14. The fact that ultra-bar occurred in the course of a downward selection of bar is interesting, though probably not significant, in view of other mutations affecting facet number which have not always been in the direction of selection.

Acknowledgments

In common with other students of Drosophila, I have had the generous help with materials and suggestions of Prof. T. H. Morgan and Drs. A. H. Sturtevant and C. B. Bridges. It is a pleasure also to express my indebtedness to Dr. Joseph Krafka, Jr., to whose untiring care of the stocks and tests the success of these experiments is largely due. I am also under obligation to Prof. A. R. Crathorne, who was kind enough to go over the method of factorial units which I have used in tabulating the data.

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Resumen por el autor Harold Cummins.
Universidad Tulane.

El papel de la voz y la coloración en la emigración primaveral de la rana y en el reconocimiento sexual.

Mediante una trampa que rodeaba casi por completo a una charca en un bosque, el autor capturó ranas pertenecientes a cuatro especies, cuando intentaban entrar en la charca para criar. La emigración tiene lugar en oleadas sucesivas, durante periodos de humedad relativa elevada coincidentes con una temperatura comprendida entre unos 41° y 52°F. El periodo de emigración se prolongó hasta cuarenta y tres dias en el caso de la rana leopardo. La intensa emigración siguió a periodos durante los cuales no se oyó el canto de las ranas en la charca o en sus alrededores. Por otra parte un aumento en la emigración no acompañó o siguió a un periodo de actividad vocal considerable. De donde el autor deduce que el impulso migratorio está regido por factores diferentes de la voz y que esta última no es un factor incitante o directivo esencial. La vista no es necesaria para la cópula y no parece jugar un papel importante en el reconocimiento sexual. El autor pudo observar algunos machos intentando aparearse con otros machos tal como si fueran hembras. Los machos normales objeto de tales intentos de cópula luchan, inflan los sacos vocales, y cantan, obteniendo de este modo la libertad. Las hembras por el contrario, ofrecen generalmente poca o débil resistencia y en la mayor parte de los casos se dejan retener por los machos. El reconocimiento de los sexos, tal como se manifiesta en el apareamiento normal, se atribuye al comportamiento diferencial de los dos sexos cuando son retenidos por un macho. Es pues un reconocimiento ulterior que depende de la reacción del macho que intenta la cópula hacia este comportamiento diferencial.

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THE RÔLE OF VOICE AND COLORATION IN SPRING MIGRATION AND SEX RECOGNITION IN FROGS[1]

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While the habits of frogs have been described by numerous writers, but little emphasis has been accorded the important phases of migration into the ponds for breeding and the method of sex recognition. Following the suggestions of Prof. Jacob Reighard, the writer carried out in the spring of 1914 a series of observations devoted to these aspects of the breeding behavior.

Work was conducted in White's Wood, near Ann Arbor, Michigan, a hardwood tract containing five small ponds, one of which was selected for intensive study. This pond is bounded on one side and one end by wooded land and elsewhere by a cultivated field which adjoins the wood. During the high water of spring the pond is about 320 feet long, with an average width of 35 feet and a maximum depth of about 3 feet. Apparatus and camp supplies were installed on March 23rd; the writer remained at the pond until May 10th.

Sex-recognition observations were made upon frogs both in the pond and in terraria. Data on migration, except for a few instances mentioned later, were obtained from the catch of a frog trap. The trap consisted of a 14-inch white-cloth fence supported by wooden stakes which made an angle of 135° with the earth on the inner or pondward side. The fence was placed about two feet from the edge of the water and extended more than two-thirds around the pond, on the side bordered by the wood and on the end and side next to the field. At intervals leaders of similar construction, but vertically supported, were

[1] Contribution from the Zoölogical Laboratory of the University of Michigan.

connected at right angles to the main fence. The lower edge of the cloth, both of fence and leaders, was imbedded in the soil and fastened with wire pins. Underneath the junction of the main fence with each leader a 2-gallon pail was sunk to the ground level and kept half filled with water. The apparatus proved to be very effective. Frogs apparently did not perceive its presence until actually in contact with the cloth. When a frog approached the vicinity of a leader but a short time elapsed before he dropped into the pail, with no chance of escape. If the frog came in contact with the main fence at some distance from a leader, he usually moved along the fence and was eventually trapped in a pail. But a less active individual might remain nestling in the angle between the fence and the ground. A careful examination of the fences and pails was made at least twice each day, morning and evening, the catch was recorded, and the frogs were transferred to terraria for observation. The fence was originally planned to completely enclose a smaller pond. Because the ice first disappeared from the larger pond, more exposed to the sun, the plan was altered, allowing only a partial enclosure of the larger pond. Future work of this sort should be done with the observation pond completely enclosed, thus ensuring the trapping of all migrating frogs.

The frog fauna of White's Wood includes the leopard frog (Rana pipiens Shreber), the wood frog (Rana cantabrigensis Baird), the pickerel frog (Rana palustris Le Conte), the green frog (Rana clamitans Latreille), the swamp tree frog (Chorophilus nigritus Le Conte), the spring peeper (Hyla pickeringii Holbrook), and the common tree frog (Hyla versicolor Le Conte). Migration data for the leopard frog, wood frog, swamp tree frog, and spring peeper were obtained, but for sex recognition work only the first two species were used.

MIGRATION

With the approach of spring, frogs desert their hibernation quarters for breeding places. Doubtless many of them hibernate in the mud at the bottoms of the same ponds where they breed,

but some winter elsewhere, perhaps in near-by bodies of water or possibly in such favorable locations on land as in masses of dead vegetation. In other locations than White's Wood the writer has found leopard frogs hibernating under conditions fulfilling all three of the above possibilities: in the mud of bodies of water where later the species was commonly found breeding, in mud and plant material in the bottoms of streams which were swift flowing and unfavorable for breeding, and, lastly, in masses of swamp grass in lowland. In White's Wood frogs might have wintered in the pond, in adjoining ponds, in springs or a creek near the observation pond, or in the adjacent wood and field. Wherever they may have been for the winter, many frogs resorted to the observation pond to breed. The questions of what incited a spring migration of those frogs hibernating away from the pond and of the conditions under which it occurred are of interest.

But little benefit can be expected from direct observations, because of the relatively small number of frogs that can be noted in their migration. Banta ('14) mentions his observation of two female wood frogs making their way to the pond where he studied the mating behavior of this species. While at White's Wood no effort was made to collect data by direct observation, the following records were made. On March 27th, at 10:30 A.M., a female leopard frog was found in the field adjoining the pond, headed toward the water. In several instances ovaries and oviducts, presumably of the leopard frog, were found 90 to 100 feet distant from the pond. Possibly these remains represented migrating frogs captured on their way by crows. Between 9 and 10 P.M. of March 31st, during a warm rain, dozens of spring peepers were captured while they were rapidly hopping toward the pond from its wooded side. Extended data on migration were obtained from the trap catches, presented below in table 1. Following the table are extracts from field notes which describe weather conditions for three days preceding the beginning of trap catches and during the period of maximum migration, including also observations of frogs appearing within the pond.

TABLE 1

Showing separately for each sex of the four species the frogs trapped between March 26th and May 7th, inclusive, also temperature and humidity records for the period[1] *(footnote, p. 329)*

LEOPARD FROG		WOOD FROG		SWAMP TREE FROG		SPRING PEEPER		DATE	TEMPERATURE, DAILY AVERAGE FOR 24 HOURS ENDING 7 A. M.	RELATIVE HUMIDITY FOR 24 HOURS ENDING 7 A. M.
Male	Female	Male	Female	Male	Female	Male	Female			
4	2			3	4	1		March 26	41.7	94
		2	1	4				27	51.9	100
								28	37.9	91
	1				2			29	49.0	87
7	21	1	1	3	3		3	30	44.6	100
1		1	1					31	41.0	87
	3				2	1		April 1	45.0	100
		1				1		2	42.1	92
								3	37.0	96
								4	30.7	48
								5	27.9	92
								6	30.0	78
								7	32.5	90
								8	28.1	86
								9	23.6	98
								10	30.4	74
								11	41.4	78
								12	37.7	62
								13	40.7	78
								14	37.3	91
1	3	1	1	1		1		15	50.5	91
1	1		2			1	2	16	44.6	100
2	1	1	1				1	17	50.4	91
	2							18	58.5	96
								19	63.1	93
								20	46.0	100
						1	1	21	33.6	94
								22	48.0	85
		1						23	45.3	90
								24	46.5	84
								25	49.9	100
1		1	1					26	62.0	94
2								27	56.1	94
	2		1					28	61.5	100
1	1				1			29	67.5	93
1								30	48.5	90
								May 1	42.9	87
								2	43.9	82
								3	51.7	88
								4	57.7	98
								5	65.4	96
								6	56.9	88
2								7	55.1	75
23	37	9	9	11	12	6	7			

Extracts from field notes, March 23rd to April 3rd, inclusive

March 23rd. The southern two-thirds of observation pond was free from ice, while other ponds in wood were entirely frozen over.

March 24th. A film of ice had frozen over the observation pond during the night. By 3 o'clock in the afternoon this film was melted with the exception of a thin strip along the west edge of the pond. In the afternoon three specimens of the swamp tree frog were observed. One of them was first noticed in the grass at edge of water; when startled it jumped into the pond, swam for a few inches and then burrowed into the mud. The other two were first observed swimming; they were caught and proved to be male and female.

March 25th. Two leopard frogs, sexes undetermined, were first noted in the grass on west side of pond. When startled they jumped into the water. Four swamp tree frogs were seen. All six frogs were observed in the afternoon as on the preceding day, the morning having been too cool for them to be active.

March 26th. In the early morning there was a heavy mist, followed in the late morning by a hard rain which lasted until about 1 P.M. At 11:15 A.M., during the rain, a single swamp tree frog croaked intermittently for about an hour. At 1 P.M. the voice of one swamp tree frog was noted; it continued for 45 minutes, when two other frogs took up the chorus. Their voices continued throughout the afternoon. All afternoon, too, the voice of the leopard frog was evident. Apparently a number of individuals were croaking. At 11 A.M. three leopard frogs were seen in the water at west edge of pond, at 1:45 four of this species, and in the late afternoon about ten individuals. None were collected.

March 27th. There was a rain throughout the preceding night. The air was cool and no frogs were seen or heard.

March 28th. There was no croaking during the morning, but for a half-hour in the afternoon a few isolated calls of the swamp tree frog were heard. The following leopard frogs were caught in the pond: at 11:30 A.M., three males and five females, which included one clasping pair; at 1:45 P.M., one clasping pair, and in the late afternoon one male and four females.

March 29th. There was no croaking during the morning until about 11 A.M., when the occasional calls of the leopard frog were noted. Occasionally through the afternoon a call of this species, of the wood frog, and of the spring peeper was heard, but there were no calls in the

[1] Temperature and humidity records were obtained through the courtesy of Professor Hussey, Director of the University of Michigan Observatory. These records were made by instruments situated at a distance of over two miles from the breeding pond and at a higher elevation. The temperature records in general correspond with readings made at the pond; no instrument for humidity records was available for use at the pond.

pond after 8 P.M. At 12:30 P.M., two clasping pairs; at 1:30 P.M., three females; at 3 P.M., one clasping pair, and at 4 P.M. one male, all leopard frogs, were captured in the pond. At 3 P.M., one male wood frog was caught.

March 30th. No croaking occurred during the preceding night and none was heard until 9 P.M. of March 30th, when the calls of the leopard frog began. In the morning a number of leopard frogs were seen in the water, none of them except one clasping pair being taken. One female wood frog was seen.

March 31st. Great numbers of unpaired wood frogs and clasping leopard frogs were noted in the pond in the afternoon. The voices of R. pipiens were first heard at 9:30 A.M.; from that time and through the night their voices were heard. Beginning at 10 A.M., the swamp tree frogs began to croak in numbers, and in the evening the voices of the spring peeper and the wood frog were added to the chorus. At 9 P.M. all four species were found at the edge of the water. The light of the lantern dazed them, for, while continuing their calls, they did not jump readily when disturbed.

April 1st. The chorus of the preceding night continued all morning. Several clasping pairs of the leopard frog were noted in the water.

April 2nd. A few swamp tree frogs croaked all day.

April 3rd. No croaking was heard until evening, when the swamp tree frog and the spring peeper began.

The numerous frogs which appeared in the pond either had hibernated therein or had evaded the trap. Both sources might have furnished individuals, the latter being possible for two reasons. All species could have entered where the fence was not continuous, and the spring peeper, also perhaps the swamp tree frog, could have adhered to the fence and climbed over. It is unlikely that the leopard frog and wood frog could have done this, for experiments demonstrated the effectiveness of the fence as a barrier for these species.

With the exception of three examples, all trapped frogs were adult and sexually mature. These instances were excluded from the records of the trap, to be recorded separately. On March 29th an immature leopard frog was trapped, on April 2nd an immature wood frog, and on April 16th an immature wood frog. Unfortunately, no examinations of the reproductive organs were made for the determination of sex, but the three were so small that they could not have been sexually mature.

Migration occurred both during day and night. For the period including March 26th through April 2nd, the time of concentrated migration, table 2 shows an equal distribution of day and night migrations, the totals including both sexes of the four species. The numbers of individuals of any one species or sex are too small to admit of further conclusions, except that night time seems to be more favorable for the migration of the wood frog and spring peeper. It appears, then, that the inciting stimulus for migration and the factors controlling the migration behavior operate independent of light and darkness.

TABLE 2

Showing, separately for each sex of the four species, the day and night distribution of migrations between March 26th and April 2nd, inclusive

DATE	DAY								NIGHT								DAILY TOTALS, BOTH SEXES, ALL SPECIES
	Leopard frog		Wood frog		Swamp tree frog		Spring peeper		Leopard frog		Wood frog		Swamp tree frog		Spring peeper		
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	
March 26	4	2			3	4	1										14
27											2	1	4				7
28																	
29										1				2			3
30	7	9			3	3				12	1	1				3	39
31			1						1			1					3
April 1										3				2	1		6
2											1				1		2
Totals........	11	11	1		6	7	1		1	16	4	3	4	4	2	3	74
	Day total, 37.								Night total, 37.								

Temperature was an important factor in the control of migration. No catches were made in the period between April 3rd and April 14th, inclusive, a time when the thermometer was low. The lowest temperature accompanied by migration was 33.6° F., and only two frogs were trapped on that date. The largest catches were made between 41° and 52° F., this range indicating an optimum temperature. No temperatures were high enough to determine a maximum.

Humidity as well played an important rôle in migration. The lowest relative humidity accompanied by migratory activity was 75 (table 1, May 7). The optimum humidity, however, was much higher, ranging between 90 and 100. It is not surprising to find a high humidity requirement in this group. From the data at hand it is difficult to ascertain the relative importance of temperature and humidity. Since in the cool period of April the humidity was at times as high as that favoring migration in warmer weather, and since migration temperatures were associated with high humidities, the inference is that migration necessitated a coincidence of favorable temperature and favorable humidity.

The duration of the migration period was much longer than would be expected. The periods in 1914 for four species were as follows: leopard frog, 43 days; wood frog, 33 days; swamp tree frog, 35 days; spring peeper, 27 days. In other years these periods might be shortened by more favorable weather conditions or lengthened by adverse circumstances. If the cool weather of April had not intervened, the periods in 1914 might have been shortened considerably. Inasmuch as the frogs were hibernating under variable conditions, it is conceivable that the duration of warm weather necessary to arouse some of them would be longer than the requirement of other frogs hibernating in readily warmed localities. Further, in localities permitting frogs to hibernate under similar conditions, perhaps the wave of migration would have an early climax and be very short. In support of the supposition that some frogs were aroused late, only one type of concrete evidence can be offered here. As late as April 27th there was taken in the trap an occasional leopard frog with the flabby edoematous appearance of individuals fresh from hibernation. We know nothing of the wanderings of frogs en route to breeding ponds. Inasmuch as the references to this point lead one to infer that the route is always a direct one and probably continuous, the results of an experiment leading to partially contradictory evidence is here recorded. On March 31st fourteen leopard frogs captured in the pond, two males and twelve females, were each marked by a white string tied care-

fully about the joint between femur and crus, then liberated in the wood about 1000 feet away from the pond. Only four individuals were recovered, the remainder probably having migrated to a pond on the opposite side of the wood. These catches were not included in the trap record. The dates of capture follow: a male, April 1st; a male, April 12th; a female, April 16th, and a female, April 18th. Reference to table 1 shows that a cool spell intervened between the trapping of the first individual and of the last three. That this fact alone was not responsible for the extended time requirement of the last three individuals is evidenced first by the return of one frog as early as the next day after liberation, and second by the migrations of other frogs in the interval.

To voice has been generally attributed the function of attracting frogs to their breeding ponds. In the species under consideration the males are provided with huge resonating sacs opening into the mouth cavities, whose presence results in a marked increase in the volume of sound. A general account of voice is extracted from Holmes ('12): "The voice of the male is louder and deeper than that of the female and is more often heard. In large frogs the notes are deeper than in small ones. The notes of frogs are more often heard in the breeding season, when they are supposed to serve the purpose of a sex call. In the summer, however, it is not unusual to hear the croaking of frogs, especially in the evening. A damp atmosphere is conducive to their song, and for this reason the voices of these animals are often heard upon the approach of a shower." The writer did not hear female leopard frogs croaking during the breeding season of 1914 except under circumstances which would permit of explanation by reflex croaking in response to tactual stimulation. Males, on the other hand, not only exhibited the croaking reflex, but also croaked of their own accord. The chorus of male toads has been studied by Courtis ('07) and Miller ('09). Their results indicate that voice in this form does serve as a sex call, but the writer's study of frogs does not support this view, at least it indicates that the chorus is not necessary to incite or direct migration.

Trap records for certain days were made after and during periods when no calls were heard in the pond. The writer is able to discriminate the voices of the White's Wood species and was at all times on the alert to hear them. The particular days cited below were selected because the night observations as well as those during the day were continuous. Thirty-nine frogs were trapped on March 30th, more individuals than were caught in any other day; in fact, this number is about one-third of the number obtained during the entire period of trapping. Table 3 presents the catch of March 30th, the twenty-four hours between 8 P.M. of March 29th and 8 P.M. of March 30th. On

TABLE 3

Showing the number of frogs of each sex of the four species migrating between 8 p.m. of March 29th and 8 p.m. of March 30th, with the times of collections from the trap on March 30th

TIME OF COLLECTION FROM TRAP	LEOPARD FROG		WOOD FROG		SWAMP TREE FROG		SPRING PEEPER		TOTALS
	Male	Female	Male	Female	Male	Female	Male	Female	
8.00 A.M.		12	1	1				3	17
1.45 P.M.	4	6							10
5.00 P.M.	3	3			3	3			12
Totals............	7	21	1	1	3	3		3	39

March 29th there was no croaking in the pond until about 11 A.M., when an occasional croak of the leopard frog was heard. In the afternoon, and continuing until 8 P.M., the voices of the leopard frog, wood frog, and spring peeper were noted, but the chorus did not continue thereafter. All frogs were silent until about 9 P.M. of March 30th.

Conversely, the catch of April 1st, while not as small as that of some other days, is interesting because of the relatively small total trapped when climatic conditions were favorable and much croaking occurred. The average temperature and humidity for the twenty-four hours ending at 7 A.M. April 1st (table 1) were favorable to migration. The total catch, three female leopard frogs, two female swamp tree frogs, and one

male spring peeper, represent frogs migrating between 9 p.m. of March 31st and 8 a.m. of April 1st. Throughout March 31st, beginning at 9:30 A.M. the leopard frogs were croaking, the swamp tree frogs began at 10 A.M., and in the evening the other two species started their chorus. All four species called during the night. Comparison may be made with the catch of March 27th, a day characterized likewise by a small catch, favorable weather conditions, and croaking, but differing in that it preceded rather than followed the day of largest total catch, March 30th. As on April 1st, the temperature lay within the optimum range and the air was saturated to 100. The catch included two male wood frogs, one female wood frog, and four male swamp tree frogs, captured in the period between the evening of March 26th and 8 A.M. March 27th. All afternoon and into the night of March 26th the swamp tree frog and leopard frog chorused, and at 11:30 P.M. the spring peeper began to call. One of the direct observations can be applied in this connection. The single female leopard frog which was picked up in the field on the morning of March 27th was making her way toward the pond despite the fact that there was no croaking.

To ascribe a directive function to voice seems to be unwarranted in the light of migrations which occur without the presence of this suggested factor. In order to perform a directive function, the chorus must be accompanied by migration, and we do not always find the two coincident. On March 30th large numbers of frogs migrated because of favorable climatic conditions; warm weather had been prolonged enough to arouse them and the weather conditions were such as to allow an overland trip. Few individuals migrated on April 1st; the small number admits of explanation on the ground that the migratory climax had passed, that a longer duration of warm weather would be necessary to arouse the frogs remaining in less readily warmed locations. The small catch of March 27th does not indicate necessarily that voice was not effective, rather that up to this date only those frogs in the most easily warmed situations had been aroused. The immediate inception of the migratory impulse must be intrinsic; physiological processes with which this

problem is not concerned (probably associated with the state of the reproductive organs, since with the exception of three immature frogs only sexually mature individuals migrated) govern the impulse, and are operative when external circumstances become favorable. Because migration is successfully accomplished without the directive influence of voice, we must look elsewhere for factors controlling the direction of migration. In such a closely related group as the salamanders there are some forms which lead a terrestrial existence at all times except during the breeding season, when they resort to water. (In the trap a number of examples of Ambystoma punctatum and several A. tigrinum were obtained.) Here as well as in other groups which are voiceless the factor of a vocal attraction is unquestionably eliminated. Neither can we explain by a vocal factor the exodus of frogs from their breeding places after the termination of the breeding season, or the emigration of newly transformed frogs. To substantiate the idea that voice is not effective, there are the results of Yerkes ('05) on auditory responses in frogs. Yerkes finds that while frogs possess a fairly well-developed sense of hearing, its function seems to be "a warning sense which modifies reactions to other simultaneous or succeeding stimuli." He does not find evidence that it serves as an independent control of motor reactions.

SEX RECOGNITION

The results of Holmes on Amphipods ('03), of Pearse on crayfish ('09), and of Reighard on the brook lamprey ('03) show that in these animals sex recognition is established through the reactions of those individuals with which the breeding act is attempted by males. That is, there is really no precopulatory recognition, rather a male may attempt union with any individual, and the reactions of that individual determine whether or not the union shall continue. Holt's observations on the dragonet ('98) and Miss Reeves' study of Etheostoma ('07) show that behavior is the criterion of recognition in these fishes. However, in these instances there is a visual recognition before the onset of the breeding act. Banta ('14), working on the wood frog, concludes

that "the color of the female may possibly be a factor and that the behavior of the female is probably a factor in sex recognition." While Banta notes that the usual procedure is the indiscriminate testing of many individuals regardless of their sex, he nevertheess postulates a precopulatory recognition established through sensory channels. This writer does not attribute any significance to the postcopulatory reactions of clasped frogs; in fact, he finds no consistent difference in the resistance of the two sexes when clasped. Miller ('09), on the other hand, finds in toads that "males cannot distinguish at sight males from females. For this reason they are continually clasping one another. They have a call of three or four notes which they utter in rapid succession when taken up between the finger and thumb, or clasped by another male. This seems to be a warning signal, for a male will release another as soon as he chirps." In an effort to obtain evidence on sex recognition, the writer observed mating reactions in the wood frog and leopard frog, both under natural conditions in the pond and under experimental conditions in terraria

In both species, pursuit under natural conditions did not differ whether the frog pursued was male or female. In this respect the observations are contrary to those of Banta, who states that "The beginning of the attempt of a male upon a female is of course not in any way different from his approach toward another male, but when he actually touches or often only nears the female his actions are usually very different, for instead of the vigor and aggressiveness of the assailant rapidly falling off, as in the case of one male approaching another, the aggressiveness is tremendously increased." In practically every instance none but moving frogs were pursued, although at a distance of a foot or less a quiet frog might be attacked, and a quiet frog if touched by a male usually was attacked, in both cases regardless of sex. A male upon which clasping was attempted sometimes only croaked when touched; if the attempt to clasp him were continued, violent struggles, croaking, and inflation of the vocal sacs were followed by dislodgment of the clasping male. Females when clasped were occasionally passive, sometimes struggled very vigorously, and sometimes struggled only a little.

Usually a male continued his efforts to clasp a female despite her struggles, but frequently he desisted, even in a few instances when her opposition was but weak.

More instructive data were obtained from observations in terraria. Some of them are presented below; with the exception of nos. 5 and 10, each observation or experiment was repeated, with results no different from the cited cases.

Observations in terraria

1. Wood frog, April 1st. The frogs of a pair were separated and placed in a large glass container with $1\frac{1}{2}$ inches of water. The male swam first in one direction and then in another, with no relation to the position of the female and without touching her. After a short time he stopped about 2 inches behind and to the right of the female. Then with a sudden jump he mounted, encountering no resistance. After being again separated, the male swam about as before. The female alternately crouched at the bottom of the dish and swam erratically. Activities of this nature followed for twenty minutes, when the frogs encountered each other snout to snout. Suddenly the male clasped the female about her head and then gradually worked backward until he secured a hold in front of her fore legs. After two minutes a sudden maneuvre placed the male in the correct position for a pectoral clasp. During this procedure neither frog croaked and the female did not resist.

2. Wood frog, April 1st. A male and a female which had not been clasping were placed in a container with leaves but no water at the bottom. With his fore legs touching her, the male crouched at the side of the female. Suddenly he jumped upon her and attempted to clasp, at the same time squawking loudly. But the female resisted, jumping up on her hind legs and turning the ventral surface upward. She succeeded in dislodging the male and he did not again try to clasp her. This clasping and dislodgment occupied not more than a half minute.

3. Wood frog, April 1st. A number of individuals of both sexes were confined in a terrarium. A male secured a clasp upon another male. The clasped male resisted, croaking and turning on his back. After a few seconds the clasping male loosed his hold.

4. Wood frog, April 2nd. Three males which had been clasping were placed in a terrarium with a male so disabled that he could not use the hind legs. After a few minutes the disabled male was clasped, and was held so for several hours.

5. Wood frog and leopard frog, April 1st. Two male wood frogs were confined in a terrarium with about twenty leopard frogs of both sexes. On the morning of April 1st, after two days in the terrarium, one of the wood frogs clasped a female leopard frog. He experienced

a little difficulty in gaining a hold upon so large a mate, but as she did not oppose his efforts, succeeded. The clasp continued for eight days.

6. Wood frog, April 1st. At 4 P.M. on this date five males and three females were placed together in a light-proof container. At 8 A.M. of the next day the container was opened, and all three females were found clasped by males. In the interval, especially at first, croaking was heard within the container.

7. Wood frog, April 1st. In a terrarium containing a number of this species one male clasped another. The clasped male resisted, turning on his back and croaking loudly. After a half minute the clasping male loosed his hold.

8. Leopard frog, March 30th. Two males which had not been clasping and one male from a pair were placed in a terrarium with a female from a pair. One of the males obtained a clasp on the female, but she struggled so violently as to succeed in dislodging the male.

9. Wood frog, March 31st. A female was introduced into a terrarium containing two clasping pairs and three single males. Within ten minutes one of the males tried to mount her; he approached from her left side, but she pushed him back with her fore leg. He persisted in attempting to mount, and after three minutes her resistance became more marked—jumping about and turning the ventral surface upward. In spite of this opposition, the male grasped her; within seven minutes he secured the usual clasp and remained.

10. Wood frog and Ambystoma, April 1st. Three single males and two females which had not been pairing and a clasping pair were placed in a terrarium with a female Ambystoma punctatum. The terrarium contained no water, only damp leaves and grass at the bottom. In a few minutes two males clasped the salamander, both with their heads directed toward her posterior end, one of them clasping her head and the other her body a little behind the forelegs. In nine minutes the third male clasped the salamander midway between the two pairs of legs. All three held tightly. When she tried to dislodge them their clasp tightened and their hind legs were braced against the body of the salamander. In forty-five minutes the third male dropped off, but in twelve minutes returned and secured a clasp in approximately the same position as before; he kept his vocal sacs inflated and croaked repeatedly. After two or three minutes he let go, the salamander writhing and struggling. At 1:05 P.M. the male which had clasped her head let go, and at 9 P.M. the one clasping back of her fore legs did likewise. During the last hours of clasping the salamander lay quietly.

11. Leopard frog, March 30th. On the evening of March 30th seventeen females and twelve males were placed in a terrarium, rather closely crowded. After an elapse of twelve hours there was only one clasping pair.

12. Leopard frog, March 31st. Two single males, a single female, and the male and female separated from their clasp were placed in a terrarium at 9:15 A.M. At 10:45 both females were clasped; at this

time the pairs were separated and the frogs returned to the terrarium. At 1:45 P. M. only one of the females was clasped.

13. Leopard frog, March 30th. Several individuals of both sexes were placed in a terrarium. When touched by a female one of the males croaked forcibly. The terrarium was crowded and the female was climbing over him.

14. Leopard frog, March 30th. When rubbed or touched, even lightly, by other males (in the same terrarium as no. 13) the response was the same as when a female touched a male.

15. Wood frog, April 1st. A clasping pair of wood frogs, three males, and two females were placed in a terrarium. A single male approached the pair, and when he touched them the clasping male croaked and warded off the intruder with his hind legs. The approaching male made no effort to clasp.

From the foregoing observations, it is evident that males attempt to clasp individuals of both sexes under experimental conditions as well as in the pond. In no. 1 the female did not resist and the male retained his clasp. On the other hand, in no. 9 she resisted strenuously, but the male succeeded in clasping and remained in spite of the resistance. In no. 2 and no. 8 the females dislodged the males. Thus there seemed to be no consistent reaction of clasped females. When males were touched by other frogs (nos. 13, 14 and 15) they croaked, and this alone seemed to be at times sufficient to frustrate further attempts of the approaching males. But sometimes, as in no. 3 both resistance and vocal remonstrance followed an actual attempt to clasp. In no. 4 the clasped male was unable to offer resistance, and the clasp in this case was retained for several hours. In agreement with Banta, there was no consistent difference in the resistance offered by the two sexes, except that males, unless experimentally disabled, always croaked or resisted, or did both, while females sometimes resisted and sometimes failed to do so. Neither was there a consistent ardor of the attacking males; sometimes, in spite of resistance offered by a female, he struggled until a hold was established; at other times the males were not persistent in their attempts upon females. But attempts on males were not continued. Pairing did not occur in all cases when frogs were placed in terraria, as in no. 11. It is significant that on the two days preceding this experiment

and on the day of the experiment only few pairs were seen in the pond, while numerous single individuals were noted. The necessity of a visual factor was eliminated by no. 6, where correct coupling occurred in the dark. The efforts of clasping frogs were not confined to their own species, or even to frogs, as no. 5 and no. 10 indicate. Holmes ('12) shows that these extraspecific claspings are even more extensive.

The variation in resistance offered by females may be tentatively explained by a gradual development of the physiological state favorable for clasping. Passive acceptance of the clasping male may be associated with the optimum development of this state, while resistance may indicate that the female is not yet ready or perhaps has already undergone the climax. In the same manner, there may be a gradual development of the clasping impulse in the male, for under natural conditions it is lost after the termination of the breeding season. The less persistent efforts to maintain a hold may be associated with a small degree of development of the impulse. And the ardor which results in the clasping and retention of the clasp on females, disabled males, salamanders, frogs of other species, etc., may be associated with the maximum development of the impulse.

Now, if males attempt clasping with both sexes of their own species, with other animals and objects, it seems that sight plays no part except to inform the male that there is something to be clasped. In the light of extraspecific pairing (no. 5 and no. 10), it seems absurd to attribute any rôle of sight in sex recognition, either on a basis of color or behavior. That sight is not even essential, that other stimuli are responsible for correct coupling, was shown by no. 6.

It has been pointed out that females will sometimes resist when clasped by males, that normal males always resist. Too, it was shown that following the resistance of the female the male might or might not continue his efforts, while in the case of males he did not persist. The resistance of the males consisted not only in struggling, but also in the inflation of the vocal sacs and croaking; in fact, attacked males did at times cause the desistance of the attacking males by only croaking when they

were touched. Apparently, then, the recognition is based upon the reaction of males. The reaction which establishes recognition is a combination of vocal remonstrance and struggling which stimulates the clasping male through tactual (and kinesthetic ?) sensations. Again, according to Yerkes ('05), sounds reinforce tactual stimuli and result in motor responses, and the sense of hearing serves as a warning sense to modify reactions to other stimuli.

SUMMARY

1. By means of a trap nearly enclosing a pond, frogs of four species (Rana pipiens, R. cantabrigensis, Chorophilus nigritus, Hyla pickeringii) were caught as they attempted to enter the pond for breeding.

2. It was found that migration occurred in waves, during periods of high relative humidity coincident with temperature ranging between about 41° and 52° F.

3. By continuous short-period day and night records of the croaking of frogs in the pond it was found that intense migration followed periods during which there was no croaking in the pond or about it and that great vocal activity was not followed or accompanied by increased migration. It is concluded that *voice does not direct* the movement of the frogs into the pond.

4. Observations in the open and numerous experiments on frogs in terraria lead to the conclusion that sight plays no rôle in the attempt of the male to clasp the female except to inform him that there is something to be clasped. *Sight was not found to be essential for correct coupling and is believed to play no rôle in sex recognition.*

5. Males were found to clasp other males as well as females. Clasped normal males struggle, inflate the vocal sacs and croak, and are always released. Clasped females show usually brief and weak resistance and the clasp is nearly always retained. *Sex 'recognition' as manifested in normal pairing thus results from the differential behavior of the two sexes when clasped, and depends on the reaction of the clasping male toward this differential behavior.*

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Resumen por los autores, Henry Laurens y Henry Daggett Hooker.
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Estudios sobre el valor fisiológico relativo de las luces espectrales.

II. La sensibilidad del Volvox a longitudes de onda de igual contenido de energía.

La sensibilidad del Volvox a la radiación de diferentes longitudes de onda e igual energía (sensibilidad a la radiación de la misma energía) ha sido investigada por los autores siguiendo dos métodos: a) la duración relativa del tiempo de presentación, y b) la velocidad relativa de la locomoción (y precisión de la orientación). Una longitud de onda de λ 494μ tiene el mayor valor estimulante, como demuestran ambos métodos. La eficacia de las otras longitudes de onda presenta una disminución gradual cuando se emplean longitudes de onda mas o menos largas. Los autores insisten sobre la necesidad de usar un espectro de igual energía en tales investigaciones. También llaman la atención acerca de la presente incapacidad para hacer comparaciones con la visión humana (normal o daltónica), es decir, con las curvas de luminosidad fotópica y scotópica (o acromática) por las siguientes razones: a) a causa de nuestra ignorancia sobre las reacciones fotoquímicas y la naturaleza de las substancias fotosensitivas, y b) porque las investigaciones efectuadas recientemente sobre la visibilidad de la radiación por el ojo normal y daltónico demuestran que el efecto máximo no depende de las longitudes de onda mencionadas en investigaciones mas antiguas.

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STUDIES ON THE RELATIVE PHYSIOLOGICAL VALUE OF SPECTRAL LIGHTS

II. THE SENSIBILITY OF VOLVOX TO WAVE-LENGTHS OF EQUAL ENERGY CONTENT

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TWO FIGURES

INTRODUCTION

The present paper is one of a series dealing with the determination of the relative stimulating effect of radiation in different parts of the spectrum. The experiments to be described were made during the summer of 1917, but due to the pressure of other duties occasioned by the war it has been impossible to prepare the paper for publication until the present time. The work as it is now presented is not complete, delay in the receipt of apparatus has prevented us from continuing the work, and a number of questions are reserved for future investigation. Incomplete as the present work is, however, it is considered inadvisable to delay publication longer. A preliminary account of the results has appeared (Laurens and Hooker, '18).

The determination of the relative visibility of radiation, or the visibility of light of different wave-lengths, consists in finding the relation between luminous sensation—'light'—and radiant energy. The sensibility of the human eye, or the visibility of the radiation, at any wave-length is the ratio of the luminous intensity, measured in light units, to the intensity of the light measured in energy units. The curve obtained is the visibility for an equal-energy spectrum.

The same general procedure can, and should, be applied to the determination of the relative physiological value of light of

different wave-lengths for other organisms. In our work the energy units are equated as described in the first paper of the series (Laurens and Hooker, '17). The 'light units' are the figures calculated from rate of movement, or time taken to traverse a certain distance, or the presentation or action time, etc. Under these conditions, the sensibility of the organisms to the various lights of different wave-lengths is proportional to the reciprocal of the 'light units.'

In our first paper (Laurens and Hooker, '17) are set forth additional reasons why it is essential that work of this sort be done with an 'equal-energy spectrum' (also Sheppard, pp 102–3).

It will not be out of place to mention in passing the work which has been done more or less recently, principally by physicists, or from the physicists' point of view, on the visibility of radiation, or luminosity at constant spectral energy. In this work the distribution of the energy in the spectrum is always taken into consideration. We wish to call attention to the contributions to this subject by Nutting ('08), Thürmel ('10), Ives ('12), Bender ('14), Nutting ('15), Coblentz and Emerson ('17), Luckiesh ('17), Reeves ('18), and Hyde, Forsythe and Cady ('18). It is with these and similar results concerning the relative stimulating value of lights of different wave-lengths for the human eye, in which the luminosity process is considered independently of the color process, that comparisons of the relative stimulating value of light of different wave-lengths for photosensitive organisms, or for photosensitive protoplasm in general, must be made.

It seems best to avoid the use of the word 'color' in the present connection, since we are dealing primarily with the objective properties of radiation. Color is a psychological fact, dependent upon the integration of physical and physiological bases. In our work on a variety of organisms we are using a physical basis to gain information concerning the physiological basis, and from a strictly objective point of view, for comparison let us say, with the human eye. To say that the physiological effect of radiation consisting of a limited number of wave-

lengths represents 'color' to a non-differentiated bit of protoplasm, is to confuse physical, physiological, and psychological qualities of radiation.

The plan of work outlined includes the carrying out of experiments with lights such as we have described, to determine the visibility of light in the different parts of the spectrum, as well as the relative effects on the size of the pupil. Furthermore, the study of the action currents of the eye of various animals when stimulated by the various wave-lengths will furnish data and information regarding the relations between the photochemical and photo-electric effects. Determinations of the relative effectiveness of the same or similar lights for different organisms will furnish a fundamental basis for comparison with the relative stimulating value, as determined by luminosity curves, for the human eye.

APPARATUS

The apparatus used to obtain the lights of different wave-lengths but of equal radiant power is described in the first paper of the series (Laurens and Hooker, '17). In addition to the twenty-three lights there listed, each 30 mμ wide, and extending from λ 420 mμ to λ 670 mμ, a white light was made equal in radiant energy content to the various spectral lights. This was used in a balanced relation to the various spectral lights, as will be described below.

A small glass aquarium (26 mm. x 26 mm. and 10 mm. deep) was made to hold the organisms under observation. For initial orientation in the beam of light, the organisms were placed in a trough made by placing two strips of celluloid parallel to the direction of the beam of light. The aquarium was placed on the stage of an ordinary dissecting microscope, with a glass plate in the stage aperture. Twelve centimeters below the level of the stage there was a ruby glow-lamp on a weak Columbia dry cell, giving a very faint illumination. This lamp was needed only when the shorter or longer wave-lengths, or the white light were being used, the organisms being clearly visible in the majority of the spectral lights. It had no demonstrable

effect, the results obtained from the lights in which the organisms were visible being the same when it was lighted as when it was not.

MATERIAL

Volvox globator was found in the spring and summer and early fall of 1917 in Mill River near New Haven. Material was collected two to three times a week, and kept fresh and cool by placing the glass vessel containing the colonies in running water in a battery jar. The colonies were dark-adapted for at least an hour before exposure to the light, the stimulating effect of which was to be tested. Only photopositive colonies were used.

EXPERIMENTAL

The reactions, orientation, etc., of Volvox to white light have been carefully observed and analyzed (Holmes, '03; Mast, '07, '11). We are not primarily interested at the present time in the problem of orientation, that is, as to whether Volvox orients directly or indirectly; nor in the question as to whether the response to light is occasioned by a change in light intensity (time rate of change) or by the continuous action of light, although our results have a bearing on these questions, particularly the latter.

The experiments may be divided into two main parts: *a*) those dealing with the determination of the presentation or action time, and, *b*) those dealing with the determination of the relative rate of locomotion.

a. Determination of the presentation or action time

By the presentation time is meant the minimum time for which the organism must be stimulated or acted upon, by a stimulus of constant strength in order that a motor reaction be elicited. For the human eye, the action time, the time required for a stimulus to produce a sensation of maximum luminosity, is a function of intensity, not of color.

Between the ocular end of the telescope of the spectrometer and the small glass aquarium there was placed a shutter with stops for 1, 0.5, 0.2, and 0.04 seconds. For exposures of longer duration than one second, the 'bulb' was used and timed by a stop-watch. The aquarium was put in place on the microscope stage, so that the band of spectral light impinged on one wall, and the white light on the opposite wall, the shutter was then closed and a Volvox colony placed in the aquarium. Being stimulated by the white light it moved toward its source, that is, into the portion of the trough of the aquarium farther away from the source of the spectral light. The white light was then screened and the colony observed for a moment or two from above and from the side to make certain that there was no horizontal movement. It was easily seen that some of the colonies, after forward motion was thus stopped, swam slowly upward, while others hung apparently motionless.

The colony was then exposed to the spectral light by opening the shutter set for the shortest exposure, or for one which it was reasonably certain would have no effect. If no reaction followed, the organism was given the same exposure again, and usually a third time, allowing adequate intervals of time and taking precautions that no fortuitous horizontal movement either in the direction of or away from the source of light was taking place at the moment of exposure. The duration of the exposure was then increased until a reaction—movement toward the source of light—was obtained. In the more effective lights the colonies would often continue to the end of the trough, although, as described, they had been exposed to the light for only the short presentation time, which was succeeded by the reaction time. Ten colonies were used for each light and each colony exposed several times and the results averaged. They are shown in table 1, column 3, the figures representing the average minimal duration of exposure below which no effect is produced.

It is now well established that in order for light to produce a definite degree of effect the time required is inversely proportional to the intensity of the light The primary photochemical

action of the light will not be effective until it has reached a certain minimum. To the production of this minimal photochemical effect a certain fixed amount of energy is necessary. The minimal duration of exposure below which no effect is produced is an indication of the physiological intensity, or stimu-

TABLE 1

The relative stimulating value of spectral lights of equal energy content as ascertained from the determination of the minimal duration of exposure necessary to produce a reaction

NUMBER	WAVE-LENGTH IN Mμ	PRESENTATION TIME IN SECONDS	RELATIVE STIMULATING VALUE	
			Reciprocal of presentation time	λ MAX. = 100
1	434.0	4.6	2.17	2.21
2	444.0	3.2	3.12	3.18
3	454.0	1.8	5.55	5.66
4	464.0	1.4	7.14	7.29
5	474.0	0.53	18.8	19.2
6	484.0	0.182	55.5	56.6
7	494.0	0.102	98.0	100.0
8	504.0	0.116	83.4	85.1
9	514.0	0.23	43.0	43.9
10	524.0	0.77	13.0	13.3
11	534.0	1.8	5.55	5.66
12	544.0	2.0	5.0	5.10
13	554.0	3.6	2.77	2.83
14	564.0	4.1	2.44	2.49
15	574.5	4.6	2.17	2.21
16	584.5	5.9	1.69	1.72
17	594.5	6.1	1.64	1.67
18	605.0	7.2	1.39	1.42
19	615.0	7.5	1.33	1.36
20	625.0	8.8	1.14	1.16
21	635.0	10.3	0.98	1.00
22	645.0	11.7	0.85	0.87
23	655.0	12.4	0.81	0.83

lating value, of the light. The relative stimulating value may be ascertained by taking the reciprocal of the presentation time, since this represents the time necessary to produce a constant quotient of change and since all the lights are of equal physical intensity. These values are shown in column 4 of table 1. For plotting, the relative stimulating efficiencies are expressed as

percentages of the wave-length of maximum efficiency. The stimulating values thus obtained are to be found in column 5, the curve in figure 1. The band of spectral light with its center at λ 494 mμ has the greatest stimulating value. The stimulating efficiency of the wave-lengths decrease rapidly and fairly symmetrically on either side of this wave-length, being the same for λ 454 mμ and λ 534 mμ.

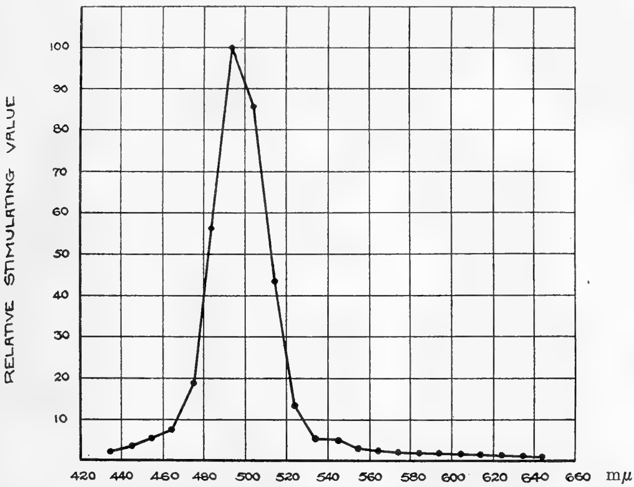

Fig. 1 The relative stimulating value of spectral lights of equal energy content as ascertained from the determination of the minimal duration of exposure necessary to produce a reaction.

Between the application of the stimulus and the initiation of the motor reaction, there is an interval, the reaction time. There is also an interval between the cessation of the external action of the stimulus (i.e., the exposure to the light) and the first evidence of the motor reaction, the latent period, during which the organism need not be illuminated. The reaction time therefore may be divided into two parts: the presentation time, which is the minimal duration of exposure below which no effect

is produced, and during which the primary photochemical effect proceeds to a certain minimal amount, e.g., by transformation of a substance already existing into another, or 'inner stimulus,' which gives rise to the impulse; and the latent period, during which a chemical process, either a continuation of the one started during the presentation time, or others, are taking place. These latter processes are not completed until at least a threshold amount of substance has been formed, or transformed, and the excitation, thus set up, conducted to the locomotor organs, or cilia, in Volvox.

Unfortunately, complete records were not made of the duration of either the reaction time or of the latent period. This is one of the points which, as mentioned above, we desire to clear up later. In our early work on the determination of the relative velocity of locomotion when the colonies were exposed to the various spectral lights, we recorded in a number of cases the time from the beginning of the exposure to the light up to the first evidence of locomotor response, that is the reaction time. We will refer to this again in the portion dealing with the rate of movement.

Hecht ('18) has obtained some very striking results in connection with the latent period portion of the reaction time of Ciona to light. He demonstrated that the latent period was a constant quantity under considerable variations in intensity, while the sensitization period (the presentation time) varied with the intensity of the light. The sensitization period, which is the reaction time minus the constant latent period, is the important factor of the reaction in connection with the intensity, since it indicates the quantity of stimulus received by the organism. The product of the sensitization period and the intensity proved to be a constant, and thus the applicability of the reciprocity law of Bunsen and Roscoe to the stimulative action of light has again been confirmed.

Mention may be made here of certain other points in Hecht's ('19 a, b, c) contributions to the analysis of photochemical reactions. In the first place, he shows good reason for believing that the photochemical process involved in the sensitization

period is of the nature of a simple reversible chemical reaction with increase of energy, taking place in a homogeneous medium. The latent period, however, involves a catalytic reaction in which the catalyst remains after the action of light, and is therefore a reaction with loss of energy.

There are two points in Hecht's work which we have not been able to make clear to ourselves, the first of these concerns the latent, the second the sensitization period, into which two parts Hecht ('18, p. 152) divides the reaction time. Although he repeatedly states ('18, pp. 162, 165; '19 a, pp. 547, 548) that under given conditions of temperature and intensity the latent period is constant, he makes the following contradictory remarks (Hecht, '18, p. 153): "It will be seen that, within limits, the shorter the exposure time (sensitization period) the longer the reaction time and consequently the latent period;" ('19 b, p. 661): "Therefore the latent period also varies inversely with the duration of the exposure."

In the above quotations exposure time and sensitization period appear to be synonymous, but in the following passage (Hecht, '19 b, p. 659) a distinction is made:

> All the experiments agree in showing that for a given intensity the reaction time varies inversely with the exposure for exposure periods shorter than the sensitization period. Exposures for intervals greater than the sensitization period make no change in the duration of the reaction time. The sensitization period may thus be defined as the minimum exposure necessary to produce the minimum reaction time.

This distinction leads to confusion. Moreover, it is contradictory to the Bunsen-Roscoe law, which Hecht ('18, pp. 155, 165, and fig. 1, p. 156) found applied to Ciona and later ('19 a, p. 548) stated "has been shown to apply to such sensitization processes."

For a given intensity there can be but one presentation time. This term is used instead of sensitization period to avoid confusion. An exposure shorter than the presentation time is subliminal. The striking similarity between the reaction-exposure curve (Hecht, '19 b, p. 659, fig. 1) and the reaction-temperature curve (Hecht, '19 c, p. 671, fig. 1) suggests a possible explanation

of the effects of exposures longer than the presentation time, which Hecht describes. In any case, the date given in experiment 9.4 (Hecht, '18, p. 153) and figures 1 and 2 (Hecht, '19 b, pp. 659, 660) are insufficient, and the experimental error is too great to justify the setting aside of the Bunsen-Roscoe law.

b. Determination of the relative rate of locomotion and precision of orientation

The same general procedure was followed in the determination of the relative rate of locomotion as was described for the determination of the presentation time. A colony of Volvox was placed in the trough of the small glass aquarium. 'The spectrometer was set to deliver wave-lengths of a certain distribution, but the aquarium screened so that the organism was not exposed to their action. By means of the white light of equal radiant power the colony was stimulated to swim to the end of the trough away from the source of the spectral light the stimulating value of which was to be tested. After, or just before, the colony reached the ends of the trough, the white light was screened and the organism, as it stopped forward motion and hung motionless or moved slowly upward, was observed by the light from the ruby glow-lamp. The colony was then exposed to illumination by the spectral light. At the first indication of movement toward the source of light a stop-watch was started, and when the colony reached the other end of the trough it was stopped. The time it took the colony to swim the distance of the trough was thus obtained. As the colony reached the end of the trough, the light being tested was turned off, and the colony observed for a few seconds as it again hung motionless or moved slowly upward. The white light was then turned on and the time it took the colony to reach the other end (the end nearer the source of white light) was taken. The reciprocal of the ratio of these two 'times' was taken as the index of the relative stimulating value of the wave-lengths in question. By taking in this way alternately the times required to swim toward the source of the white light and toward the source of the spectral light, the stimulating value of

which was being tested, the influence of change in 'physiological condition,' or of an increase or decrease in speed of locomotion and of precision in orientation, in successive tests, was eliminated.

A colony was usually given five trials, in each direction and the respective times taken to swim toward the spectral light averaged, as were those taken to swim toward the white, and the ratio between the two rates taken. From four to sixteen colonies were thus given trials in each light.

In the results obtained from the determination of the presentation time the reciprocal of this value was taken as the stimulating efficiency of the light. In comparing the relative rate of locomotion, we likewise take the reciprocals of the time required to travel a certain distance.

In table 2 the ratios of the rate of locomotion toward the source of the spectral lights to the rate of movement toward the white light are listed in column 3. The relative stimulating values of the various spectral lights calculated by taking the reciprocals of these ratios are listed in column 4, and as percentages of λ maximum in column 5. The curve is shown in figure 2.

An extensive series of tests was also made of the stimulating value of the various wave-lengths while the colonies were exposed at the same time to the white light and to the spectral light, the particular light whose stimulating value was being tested impinging at right angles on one wall of the aquarium, the white light on the opposite wall. The speed of locomotion to the first fifteen of the spectral lights while the white light was also burning was then ascertained, and the speed of locomotion to the white light alone, as above, and the ratio again taken. These values are shown in column 6, table 2; the reciprocal of them, or the relative stimulating value, in column 7, and the values computed by regarding λ maximum as 100 in column 8, which values have also been plotted (fig. 2). In the region of the spectrum of highest stimulating effects the values are not very different from those obtained for the spectral light alone (columns 5 and 8). The reason for this is probably that the stimulating effect of the wave-lengths of greatest efficiency, and of those in the immediate neighborhood in the spectrum, is relatively so great

that the stimulating influence of the white light, though of equal radiant energy power, does not, or cannot, influence the organisms. But in the regions of the spectrum of wave-lengths of less stimulating effect, the influence of the white light reveals itself,

TABLE 2

The relative stimulating value of spectral lights of equal energy content as determined from the relative rate of locomotion

NUMBER	WAVE-LENGTH IN $m\mu$	SPECTRAL LIGHT ALONE			SPECTRAL LIGHT VS. WHITE LIGHT		
		Rate of locomotion $\frac{\text{spectral light}}{\text{white light}}$	Relative stimulating value		Rate of locomotion $\frac{\text{spectral light}}{\text{white light}}$	Relative stimulating value	
			Reciprocal of rate of locomotion	λ max. = 100		Reciprocal of rate of locomotion	λ max. = 100
1	434.0	0.86	0.116	76	1.02	0.098	64
2	444.0	0.82	0.122	80	0.92	0.109	72
3	454.0	0.78	0.128	84	0.79	0.127	84
4	464.0	0.74	0.135	89	0.77	0.130	86
5	474.0	0.72	0.139	91	0.73	0.137	90
6	484.0	0.69	0.145	95	0.69	0.145	95
7	494.0	0.66	0.152	100	0.66	0.152	100
8	504.0	0.71	0.141	93	0.69	0.145	95
9	514.0	0.75	0.133	88	0.75	0.133	88
10	524.0	0.79	0.127	84	0.80	0.125	82
11	534.0	0.85	0.118	78	0.89	0.112	74
12	544.0	1.02	0.098	65	1.07	0.093	61
13	554.0	1.11	0.090	59	1.22	0.081	53
14	564.0	1.17	0.085	56	1.41	0.071	47
15	574.5	1.28	0.078	51	1.70	0.059	39
16	584.5	1.36	0.074	49			
17	594.5	1.51	0.066	43			
18	605.0	1.54	0.065	42			
19	615.0	1.60	0.063	41			
20	625.0	1.63	0.061	40			
21	635.0	1.64	0.061	40			
22	645.0	1.79	0.056	37			
23	655.0	1.79	0.056	37			

becoming relatively stronger and stronger, so that orientation and locomotion toward the source of the spectral lights are progressively made less precise and retarded, with the result that the ratio of the rate of locomotion to spectral light as compared with the rate to white light becomes greater and greater. The

influence of the white light thus relatively increased until its effect was practically equal to that of λ574mμ (light no. 15), the colonies showing an almost equal tendency to proceed to one or the other source. When exposed to the influence of the white light and to that of wave-lengths greater than λ574mμ, the white light had an increasingly greater stimulating effect as the wave-lengths became longer so that the organisms moved toward the

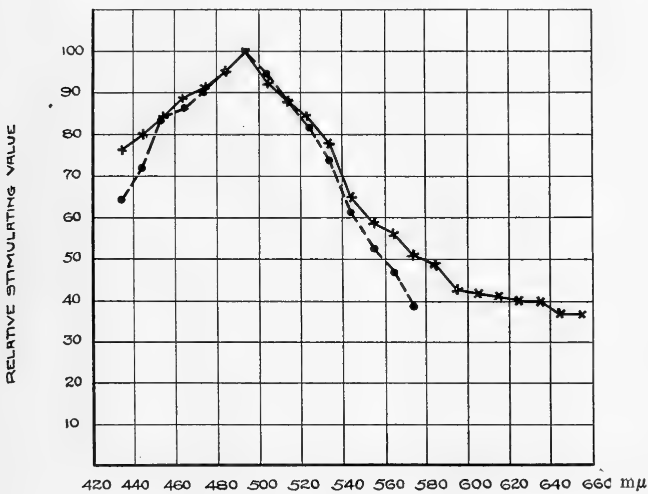

Fig. 2 The relative stimulating value of spectral lights of equal energy content as determined from the relative rate of locomotion. x——x——x, spectral light alone; •- - - -•- - - -•, spectral light vs. white light.

white light. No further record was taken of the relative rate of locomotion.

The curves show that λ494mμ is the wave-length of maximum stimulating value, as was found by the method of determining the presentation time, and that the relative stimulating effect decreases by degrees toward both ends of the spectrum. The intensity of the spectrum used, however, was so great that all of

the wave-lengths tested have a stimulating effect. With this high-intensity spectrum it will be interesting to continue the experiments into the longer and shorter waves. This it is planned to do as soon as practicable.

As mentioned earlier, the reaction time was also taken in certain cases. That is the time that elapsed between the moment of exposure of the organism to the light, and the first indication of a locomotor response, or a swimming movement, toward the source of the light. The results of computing the ratio of the reaction time in the spectral light to the reaction time in the white light show that the most effective wave-lengths ($\lambda 494 m\mu$) are the same as ascertained from the determination of the presentation time and of the rate of movement. The relative effectiveness of the various lights, due to the incompleteness of the records, is, however, not clearly shown.

We have used the time taken to traverse a certain fixed distance as an index of the relative stimulating efficiency of lights of different wave-length. Although Volvox orients fairly precisely, the colonies deflect somewhat, traveling in a wave-like course, up and down or from side to side. This deflection is less when the colonies are swimming toward the source of the lights of greater stimulating effect. In other words, the degree of deflection varies with the stimulating efficiency of the light. We did not note any consistent change in the precision of orientation or of the rate of movement in the five tests given each colony.

The relative time taken to swim a certain distance was not, however, due merely to the amount of deflection or precision of orientation, but also to the actual rate (strength) of swimming. It was easy to see in the lights of low efficiency that the colonies loafed along, now and then speeding up a little to slow down again, and so on. These variations in the energy output, or the speed of swimming movements, being irrespective of the distance which they had traversed or as to how near they were to their goal. The conclusion seems justified, therefore, that the rate of locomotion, as well as the precision of orientation, is dependent upon the relative stimulating efficiency (or physiological intensity) of the lights, those of greater efficiency causing

a greater chemical change or transformation resulting in a greater expenditure of mechanical energy.

Holmes ('03, p. 323) described the swimming of Volvox toward a source of light as follows:

> It was found that, as the Volvox traveled toward the light, their movement was at first slow, their orientation not precise, and their course crooked. Gradually their path became straighter, the orientation to the light rays more exact and their speed more rapid. After traveling over a few spaces, however, their speed became remarkably uniform until the end of the trough was reached. If the light is so intense that one end of the trough is above the optimum intensity of illumination, the speed of the Volvox is decreased as it approaches this optimum where it finally stops.

In our experiments the distance to be traversed was only 26 mm. We observed that the rate of locomotion became uniform, as Holmes describes, but the organisms always went the whole distance, so that the effect of intensity on the rate of movement which he described is not in evidence. We have, however, often observed that the rate of movement can be from the first relatively very rapid, one might say, explosive, as if the colony had been catapulted or had bounded away in the direction of the source of lights. The impression was gained that in many cases the velocity of movement slackened a little after the first outburst of speed, and that the colony settled down to a uniform rate which was held until the end of the trough was reached. This type of movement was only to be observed in the reactions to the lights of greatest stimulating efficiency. Its rate was not measured, and we are not sure that it can be measured, although it is possible that by dividing the distance by transverse lines it may be done. It was also observed with great clearness in the experiments on the determination of the presentation time. In these experiments, particularly in the responses to the most efficient lights, the Volvox would often continue to the end of the trough, although, as above mentioned, the exposure lasted for a very short time and was followed by the latent period. In many cases, however, the colony would only proceed a short distance. This sometimes happened even in those cases where the first movement was of the explosive nature described, the colony stop-

ping its forward movement and hanging apparently motionless or swimming slowly upward.

The investigation of the question as to whether the rate of locomotion, particularly in the lights of high stimulating effect, is uniform throughout the course or whether there are variations in the rate of locomotion which can be correlated with the distance already gone, the length of exposure, the relative proximity to the source (intensity), etc., is one which is reserved for future investigation. Some interesting data may thus be obtained, e.g., from the comparison between the average rate for the whole distance, or period, with the rates at different parts. In other words, the determination as to whether the rate is constant for the whole period or variable, and thus whether the curve expressing the average rate is a straight line or a curve.

DISCUSSION

There is no doubt that light stimulates nerve-endings through a photochemical reaction, the stimulation being mediated by photoreceptors, not necessarily structurally defined, but sensitive to photochemical change in the substance with which they are in contact. The effect of light is therefore due not to light directly, but to the chemical changes which it causes, these changes involving the formation of a substance or of substances which, according to mass action and reaction velocity, act as 'inner stimuli.'

Although the effect of light is due to the mass action of the chemical compounds which it produces (the photochemical reaction product), it is not assumed that the rates of the photochemical reactions themselves follow the simple law of mass action, since the rate is controlled by the amount of the light energy absorbed per unit time, and not by the actual number of molecules present (Sheppard, pp. 211, 217 ff).

The photochemical process is fundamentally an electric one, in that there is a raising of potential, due to ionization, as the immediate effect of light (electrolytic dissociation is governed by the law of mass action). An increase in permeability, resulting

from stimulation, does away with the polarized condition, by allowing the two layers of ions to mix freely, and the condition of excitation spreads or is propagated.

We prefer to leave to later development and consideration the exact relations between ionization and permeability ('cause and effect'), and at this time merely call attention to a few matters of rather general nature and import, as they bear upon our subsequent work.

The study of the relative stimulating effects of lights of different wave-lengths on organisms will undoubtedly give information concerning the nature of the photochemical changes involved in light reactions. Any particular chemical reaction is produced by a certain group of wave-lengths only, so that the possibility is presented of distinguishing between light of different wave-lengths. A study of the stimulating effect of various wave-lengths on organisms, combined with radiometric and spectrophotometric examination, showing the degree of 'energy' as well as 'light' absorption of solutions or extracts of the organisms, and of the 'visual purple' of some of the invertebrates will be of interest and value.

Although there is a wave-length which is most effective in action on a given photochemical substance, the same wave-length may be maximally effective for more than one substance. Therefore, the fact that the same wave-lengths may be found equally effective for two species of organisms does not per se signify that the photochemical substances or reactions are for that reason the same. This invalidates the value of comparisons which are made, on this basis, between the photochemical substance in a certain organism and the visual purple in the amphibian, mammalian, or human eye. The fact that a certain organism shows a maximum sensibility to certain wave-lengths which happen to be the same as those which cause most rapid bleaching of visual purple, and which are maximally absorbed by it may be merely fortuitous, and have no fundamental significance. How otherwise can it be explained that in certain organisms which are nearly related the wave-lengths of light of maximal stimulating efficiency are quite different, while in many that are

only distantly, if at all, related, the wave-lengths of maximal stimulating efficiency are practically the same? This does not mean that we cannot compare the effects of lights of different wave-lengths on photosensitive protoplasm with the effects on the photosensitive substance in the eye of man, as indicated by their respective relative stimulating values.

In this connection attention is called to the objections stated by Loeb and Wasteneys ('16, p. 224; Loeb, '18, p. 102) against the drawing of conclusions from comparisons made between the sensations of brightness in color-blind human beings and the wave-lengths of maximal stimulating efficiency for lower organisms to the effect that these lower organisms are therefore also 'color-blind.' The two photosensitive substances, visual purple and the photosensitive substance in the organism in question, are merely affected in a similar way by the same wave-lengths.

We do not wish to seem hypercritical, but feel that attention should also be called to the probability that the determinations of the wave-lengths of maximal efficiency in bleaching visual purple, and of those maximally absorbed by it, will be found to be erroneous. The work of Trendelenburg ('04) is far from perfect, particularly in the application of the energy corrections and in the determination of the region of maximum absorption (Garten, '06, '08), as indicated by the shifting of the relative absorption values. Furthermore, Henri ('11) has ascertained the threshold energy, the bleaching effect on visual purple, and the amount of light absorbed by it at various wave-lengths with due consideration to the distribution of energy in the spectrum. These three factors all follow the same course with maxima between $\lambda 520 m\mu$ and $\lambda 500 m\mu$. Also Bender ('14, '16) has recently found that the luminosity curve for the totally color-blind eye and for the peripheral field of the normal, dark-adapted eye has a maximum between $\lambda 515 m\mu$ and $\lambda 520 m\mu$.

Perhaps importance may be attached to the fact that the chemical processes in the retina are assumed to be pseudoreversible reactions, while there is as yet no positive evidence that any other reactions involving photosensitive protoplasm are of this type. Furthermore, visual purple is an optical sensitizer, al-

though we do not know whether the products of its change themselves stimulate the receptors or whether they act catalytically.

It seems to us justifiable to conclude that the influence of different wave-lengths, as far as the chemical reactions which are associated with the action of light are concerned, are fundamentally the same for animals and plants. This is all that we understand that Loeb claims when he speaks of the "identity of heliotropism in plants and animals," viz., that the reactions of plants and animals are both due to the action of light on the photosenstitive substances, resulting in transformation. For different organisms (plants and animals) there are different wave-lengths which have a maximum of stimulating effect. This signifies that there is a difference in the photosensitive substances, photochemistry telling us that the most efficient wave-length varies with the nature of the photochemical substance.

We find it difficult to understand just what Mast ('17, p. 522) means when he says, "the reactions are not wholly dependent upon wave-lengths, for while there is clearly a region of maximum stimulating efficiency in the spectrum, stimulation is not confined to this region and the stimulating effect of the wave-lengths on either side of it can be made greater by simply increasing their intensity."

It is found in plants and animals that certain wave-lengths have a maximal stimulating effect as compared with the effect of other wave-lengths. It stands to reason, of course, that this effect is due to the energy of radiation (i.e., light and heat effects, which together make up the stimulating value of the wave-lengths in question). For different wave-lengths to produce the same effect would require that the amount of energy absorbed by the photochemical substance would be the same in all cases (that is, to have the same relative penetrating power). Therefore it follows that by increasing the intensity of any wave-length we would increase its stimulating effect. It is thus possible that if the entire series of wave-lengths be made increasingly intense or greater in radiant power beyond a certain limit, a considerable portion of the spectrum in the neighborhood of the wave-lengths of maximum stimulating efficiency at a lower in-

tensity would be found to be equally efficient as stimuli, because the absolute maximum effect of the wave-lengths of greatest stimulating effect has been reached at a lower intensity of the spectrum, while that of the other wave-lengths was below their possible maximal effect, or because of the influence of some limiting factor.

It is probably also true that if the absolute intensity of an equal-energy spectrum be decreased, certain wave-lengths, which at a greater intensity have a relatively weak stimulating efficiency, will, as the intensity is decreased, have finally none at all, and this will spread and thus involve more and more of the spectrum. But the wave-lengths of maximum effect will remain the same for all intensities.

This is of course pure assumption. We know of no work on the influence of the relative intensity of the spectrum on the location of the wave-lengths of maximum stimulating efficiency for other photosensitive protoplasm than the human eye. But a low-intensity spectrum has for the human eye a region of maximum stimulating efficiency (achromatic scotopic luminosity curve) nearer the blue end of the spectrum than a high-intensity spectrum (photopic luminosity curve), and its luminosity curve is similar to that of the totally color-blind under all intensities (Parsons, pp. 189, 209), as well as to that of rod vision (peripheral vision) under high illumination (according to Bender, '14, figs. 2 and 4), the curve for the peripheral retina coinciding with that of the foveal visibility curve of totally color-blind persons, with maxima at $\lambda 515 m\mu$. (The question of the quantitative and qualitative differences between peripheral vision and scotopia on the one hand and central vision on the other, either need revision or reinvestigation. Parsons, pp. 71–72.)

Now it is a question, as above indicated, whether anything comparable can be found in the sensibility to different wave-lengths in lower organisms. Can we, by varying the absolute intensity, keeping the spectrum equal in energy throughout, shift the region of maximum stimulating efficiency? Probably not. But it is a question well worth considering and investigating. If the luminosity curve for the peripheral retina (normal rod

vision) coincides with the curve of visibility for totally color-blind persons (isolated rod vision), this latter being the same as the achromatic scotopic curve, we conclude that the rods are always stimulated in the same relative degree. High and low intensities produce the same relative effects as to wave-lengths. Applied to lower organisms, we conclude that there is nothing in the sensibility to light of different wave-lengths which would lead us to assume anything comparable to the photosensitive substance in the cones, and that no matter with what intensity of the spectrum we work, we will always obtain the same relative sensibility wave-length curve.

If a substance is sensitive to light of a particular wave-length, it must absorb this light and show an absorption band in the region in question, the absorption spectrum of a chemical system being intimately connected with its photochemical behavior. Light waves are absorbed in ponderable media by particles capable of a free period of vibration. Vibrations not synchronized to these produce only forced vibrations of the particles and would hence be only slightly absorbed.

In a heterogeneous mixture, if the substances do not interact to form a new combination, the light absorption of a mixed solution will be equal to the sums of the absorptions which the components would exert separately. That is, when chemical interactions are excluded, the behavior of summed absorptions are purely additive.

The photochemical substances in organisms are probably heterogeneous, comprising a number of different substances, so that a number of different groups of wave-lengths are absorbed, producing, by resonant vibration of different rates, chemical reactions, resulting in photochemical products. That is, in the formation of particular photochemical products, vibrations (molecular, atomic, or electronic) of a certain rate are excited by resonance, with excitation as the result of the energy set free.

For recent applications of this chemical and physical conception involving absorption, vibration and resonance in theories of color vision, see the papers by Houstoun, '16; Guild, '18, and Troland, '16 and '17.

It does not seem possible at the present time to assign a satisfactory explanation to the maximal stimulating effect of light of a particular wave-length. The following considerations are, however, of interest. The effect of the light is a result of its absorption by the photosensitive substances. Only the light which is absorbed is chemically active, though all of the rays absorbed are not necessarily active in producing chemical change. When light is absorbed, the amplitude of the vibrations is a maximum when the free periods of the vibrating particles coincide with the period of the incident light.

The wave-lengths of maximum stimulating efficiency owe their action to maximum absorption and to the hypersensitivity of the photosensitive substance to the influence of these particular rays.

But absorption alone cannot be used as a measure of physiological action, because it depends upon the kind of processes initiated by the transformation of the absorbed energy (Bovie, '18, p. 253). The kind of processes initiated are dependent upon the nature of the original photochemical substance.

SUMMARY

1. The sensibility of Volvox to radiation of different wave-lengths but of equal energy (sensibility to radiation at equal energy) has been investigated by two methods: (*a*) the relative duration of the presentation time, and (*b*) the relative rate of locomotion (and precision of orientation).

2. Wave-length $\lambda 494 m\mu$ is found by both methods to have the highest stimulating value. The efficiency of the other wave-lengths show a gradual decrease as shorter and longer wave-lengths are tested.

3. The necessity of using an equal-energy spectrum for such work is emphasized.

4. Attention is directed to the present inability of making comparisons with human vision (either normal or color-blind), that is, with photopic and scotopic (or achromatic) luminosity curves: (*a*) because of the lack of sufficiently exact work on lower organisms; (*b*) because of our lack of knowledge regarding photo-

chemical reactions and the nature of the photosensitive substances, and, (*c*) because recent work on the visibility of radiation for the normal and color-blind eye shows that the maximum effect is not at the wave-lengths indicated by earlier work.

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Resumen por el autor, F. B. Sumner.
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La variación geográfica y la herencia mendeliana.

En ocho lotes de individuos del ratón Peromyscus maniculatus, procedentes de diversas partes de California, he encontrado el autor diferencias locales significativas en lo referente a la longitud media de la cola, pié, oreja, pelvis, fémur y cráneo, anchura de la raya caudal dorsal, color del pelaje, pigmentación del pié y número de vértebras caudales. Animales pertenecientes a lo que ordinariamente se considera como la misma subespecie, pueden diferir considerablemente en diferentes localidades. Para un carácter determinado el número de variaciones no coincide con el de lotes. En determinados caracteres y en cierto grado, puede observarse una variación gradual geográfica y climatológica en estas variedades locales. El orden de gradación coincide bastante bien con ciertos caracteres, aun cuando esto no puede aplicarse a todos ellos. Los coeficientes de correlación indican que los caracteres que varían juntos, en sucesión geográfica, pueden variar o no variar juntos en un lote local y vice-versa. De este modo, circunstancias especiales que actúan de un modo local deben causar la modificación simultánea de partes que ordinariamente no varían juntas. Las diferencias entre estas razas locales no se comportan en los cruzamientos como simples factores mendelianos, aunque la teoría de los "factores múltiples" se invocaría indudablemente en este caso por muchos geneticistas. El autor ha comparado las desviaciones tipos de varios caracteres en las generaciones F_1 y F_2. En la mayor parte de los casos el ligero aumento en la variabilidad se presenta en la generación F_2, aunque en una considerable minoría los numeros obtenidos son próximamente los mismos en las dos generaciones, o las condiciones pueden aun invertirse. Los experimentos mencionados arrojan incidentalmente luz sobre algunas diferencias sexuales secundarias inesperadas.

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GEOGRAPHIC VARIATION AND MENDELIAN INHERITANCE

FRANCIS B. SUMNER

SEVEN FIGURES

INTRODUCTION AND SUMMARY OF RESULTS

In several previous papers[1] I have reported upon the results of a biometric and genetic study of the geographic races of a single species of deer-mouse (Peromyscus maniculatus) found within the State of California. The present paper, like the former ones, is merely a report of progress. I shall here discuss the more important additions to my previous findings, based upon the work of the last two years. I shall, however, make only incidental reference to certain prominent phases of this work, such as the study of the various color mutations which have appeared and the field studies of environmental conditions. These will be embodied in separate papers, to be published before long by Mr. H. H. Collins and myself.

Thus far, collections sufficient for statistical purposes have been made in eight different localities and minor collections at five or six others. Over three thousand animals, wild and cage-born, have been subjected to measurement. Only wild specimens, unmodified by captivity, have been dealt with in computing the differences between the various races here discussed.

The characters chosen for study have been, as far as possible, ones which are capable of quantitative expression. As emphasized in earlier papers, the methods employed have been standardized, so as largely to eliminate differences due to irrelevant circumstances and to 'personal equation.' What these characters are will appear in the ensuing pages. Most of them have

[1] American Naturalist, November, 1915; *id.*, March, 1917; Genetics, May, 1917; Bulletin of the Scripps Institution, No. 3, October 19, 1917; American Naturalist, April–May, June–July, August–September, 1918.

been dealt with in previous reports. A further character of great importance, which I plan to study quantitatively, is the color of the pelage, but no data from this source will be considered in the present paper.[2]

I shall not regard it as necessary here to discuss either the technique followed in making the measurements or the methods employed in the subsequent statistical treatment. Some of my former papers have covered this ground sufficiently well for present purposes.

To reverse the conventional procedure, I propose here to summarize and discuss my results in advance of the more detailed account. This will serve to make clear at the outset the purpose of the paper, and will perhaps render the detailed statements more intelligible to such readers as may stray beyond this introduction.

To proceed with this general discussion, significant racial differences have been found in respect to the mean length of the tail, foot, ear, pelvis, femur, and skull, the width of the dorsal tail stripe, the color of the pelage, depth of pigmentation of the foot, and number of vertebrae in the tail. There are not, however, for any one character, as many grades to be distinguished as there are localities from which collections have been made. For example, I have thus far found only six distinguishable grades in respect to tail length and only four or five in respect to foot length.

Local collections from different parts of the ranges of the same 'subspecies,' as thus far recognized by systematists, have been found to differ considerably. Thus, the two extremes, in respect to ear length, are presented by the Berkeley and the La Jolla collections, both of which are generally assigned[3] to the subspecies 'gambeli,' while the subspecies 'rubidus,' as regards several characters, presents at least three well-marked grada-

[2] Mr. H. H. Collins is preparing a paper which includes color analyses made in accordance with a method which we have developed jointly.

[3] Stephens (California Mammals, San Diego, 1906); Osgood (Revision of Mice of the American genus Peromyscus, Bureau of Biological Survey, Washington, 1909); Grinnell (Distributional List of the Mammals of California, Proceedings California Academy of Sciences, August 28, 1913).

tions, as we pass southward from Humboldt to Sonoma County. In fact, the collection from Duncan Mills, near the mouth of the Russian River, is about intermediate between 'gambeli' and the more extreme 'rubidus' type of Humboldt County. Nevertheless, it is a significant and striking fact that the 'gambeli' mice from the vicinity of Calistoga, in Napa County, much more closely resemble those from La Jolla, 500 miles distant, than they do the 'rubidus' from Duncan Mills, only twenty-seven miles away.

To a certain extent, and for certain characters, the gradations here considered follow a geographic—and also a climatic—sequence. The degrees of difference in the characters are, however, by no means proportional to the geographic intervals between the 'races,' and there are other incongruities which greatly complicate the situation. Thus, while there is strong evidence for some sort of causal relation between these local differences in the mice and some element or elements in the physical environment, it is not possible to state with confidence at present just what these elements are nor how they operate to modify the organism.

The order of gradation for certain of the characters agrees with that for certain others. Thus the order for tail length, foot length, and tail stripe is similar, though not identical, while the order for general color and that for skull length each presents some significant points of resemblance to that for the other characters just named.

Certain characters, on the other hand, present a totally different arrangement. Thus ear length and the length of the pelvis (innominate bone) offer gradations which bear no relation to one another or to those of the other characters. Again, it must be emphasized that there are well-marked differences even among those characters which approach one another most nearly in their order of arrangement. In respect to tail length, for example, the Berkeley and Victorville races do not present significant differences, while in regard to the width of the tail stripe they stand far removed.

Coefficients have been computed, showing the extent of the correlation among certain of these characters within each single race. This, needless to say, is another way of ascertaining

whether or not the respective characters tend to vary together. Of course, the size of all the parts of the body is correlated with the general size of the animal. Thus, larger animals have longer tails, feet, etc. Also, in a series of mixed size, any parts which are correlated with body size are necessarily correlated with one another. But this is not the sort of thing which concerns us here. Of chief interest are the correlations which are found to exist between two characters, when the element of body size has been eliminated. For this purpose, we may either parcel out our animals into groups having approximately the same body length, and determine our coefficients within each of these, or we may employ material of varying size and resort to the method of 'multiple correlation.' For certain reasons the former method has been employed exclusively, at least for such characters as vary with the size of the body.[4]

Thus proceeding, I find that there is a small, though probably significant, positive correlation between the length of the tail and that of the foot and skull, and a less certain one between tail and ear, while the length of the foot likewise appears to be positively correlated with that of the pelvis.

The tail-to-body ratio is negatively correlated with body length; i.e., longer mice have relatively slighter shorter tails. On the other hand, there is probably no correlation between body length and the relative width of the dorsal tail stripe (ratio of arc to entire circumference of tail).

The number of tail vertebrae is positively correlated with the relative, but not with the absolute, length of the tail. In other words, animals, large or small, which have relatively long tails possess, on the average, a slightly greater number of vertebrae. But larger animals have no more vertebrae than smaller ones, despite the greater absolute length of the tail. In any case, the length of the tail is chiefly determined by the size of the individual vertebrae, rather than by their number.

[4] Certain other characters, such as the relative length of the tail (regarding it as a percentage of body length), the relative width of the tail stripe (expressed as a percentage of the circumference of the tail), and the number of vertebrae are so slightly correlated with the general size of the body that correlations have been computed directly in populations of mixed size.

The width of the tail stripe (relative, as defined above) appears to be not at all correlated with relative tail length. The mean coefficient actually obtained, which is probably not significant, is slightly negative. This is surprising when we consider that the two characters in question follow much the same order in their degree of manifestation among the various geographic races, and that when these local collections are thrown together and treated as a single population, a fairly high positive correlation is found to obtain.[5]

Thus, characters which vary together, when geographic sequence is considered, may or may not vary together within any single local collection, while, conversely, characters which are correlated within these various local populations may or may not be found to have undergone concomitant modification, when we pass from one locality to another.

These relations raise the question whether the interracial differences in the mean values of various characters belong to the same type as the intraracial or individual differences. By belonging to the same type I mean having the same sensible properties, and the same mode of hereditary transmission. I think that the ensuing discussion will make clear that the sensible properties are the same, so far as inspection reveals, and that the behavior in heredity is probably likewise the same in the two cases. As to their respective causes, on the other hand, we know too little at present regarding the causes of variation in general to draw any very useful distinctions upon that basis. It would seem obvious, however, that special factors, operating locally, must be responsible for the simultaneous modification of parts

[5] A rather obvious explanation of this apparent contradiction suggests itself here, which, however, I am certain is not the correct one. It might be supposed that an actual positive correlation between these two characters exists within each of the local populations, but that this is too feeble to be appreciable, owing to the limited variability of these populations considered separately. It need only be pointed out that the standard deviations of the local collections, taken singly, are more than half (55 to 80 per cent) as great as those of the mixed assemblage which results when the data are combined. (This has been done for four races only.) Significant positive coefficients of correlation might, therefore, reasonably be expected within each local race, so far as the extent of variability is concerned.

which do not ordinarily vary together. I shall return to this point presently.

It must be borne in mind that all of the differences which I have dealt with between the local races relate to the average condition, and do not hold constantly for every individual of the groups under comparison. In fact, a large proportion of the individuals belonging to two adjacent groups might be placed indifferently in either, without the transfer being detectable by any test known to me. It is only the most widely separated collections, e.g., those from Humboldt Bay and the Mohave Desert, which differ so much in respect to certain characters that the frequency polygons for these last do not overlap more or less broadly. Between such a condition of distinctness as this and one of practically complete identity we have many gradations. In some cases it is only by the comparison of probable errors that we are enabled to say whether or not two collections differ significantly in respect to a given character.

It must be remembered, too, that the local 'races' with which I am dealing are highly artificial groups. My collections are simply samples, taken at various arbitrarily chosen points from a perfectly continuous population. Whether or not these local differences in the average or modal condition would be completely bridged by collections taken at stations sufficiently close to one another remains problematic. It would seem almost inevitable, however, that interbreeding would lead to such a complete continuity, at least in the absence of some sort of geographic barriers. For mice of even the most widely separated of these races appear to be fully fertile inter se.

It appeared early in the course of these studies that the various racial differences were hereditary. The races 'bred true,' so far as could be detected by the methods employed and allowing for certain abnormalities of form to which all of the races were subject when reared in captivity.

I have also shown that the variations within each local race—or some, at least, among them—are rather strongly hereditary. As stated in an earlier paper, the parental-filial correlation in respect to tail stripe and to relative tail length averages, in each

case, about 0.3.[6] This means, as ordinarily interpreted, that in each local race part of the variability is hereditary and part non-hereditary, this last component being regarded as 'somatic' in origin.

One essential feature of these geographic races remains, however, to be examined somewhat further. It has been shown that the shifting of mode by which one 'race' arose historically from another must have involved the simultaneous shifting of a considerable number of different modes. And this occurred even among characters which, in their every-day inheritance, do not seem to be linked together to any appreciable extent. Moreover, characters (e.g., foot and pelvis), which appear to be correlated positively in the individual, appear in some cases to have been modified in opposite directions in the course of phylogeny.

I have pointed out in earlier papers that in respect to both coat color and the width of the dorsal tail stripe a general climatic sequence is discernible among these races, and this conclusion appears to be borne out, on the whole, by the additional data presented below. I have also called attention to the agreement between my own findings in this regard and those of various mammalogists and ornithologists, who have recognized the existence of an increase in pigmentation pari passu with an increase in the atmospheric humidity of their habitat. If we consider only the coastal stations from San Francisco Bay northward (Berkeley, Duncan Mills, Fort Bragg, Eureka), which probably present a graded series in respect to both temperature and atmospheric humidity, we find likewise a similar gradation in respect to the mean width of the tail stripe and the mean length of the tail, foot, and ear. The suggestion lies close at hand that we have to do with some more or less direct influence of environment, which, in the course of time, has modified the hereditary characters of the animals dwelling at these various points.[7] Perhaps the four characters just named have undergone simultaneous modification by some single external agency, and this

[6] American Naturalist, June–July, 1918, p. 294.

[7] This supposition might, of course, be expressed in such terms as would exclude the 'inheritance of acquired characters.'

might be held to account for the parallel modification of parts which ordinarily do not vary together.

Now, I regard it as highly probable that such racial differences as relate to pigmentation have been produced in some way by environmental agencies. And our problem would doubtless be simplified if we could regard the other differences named as having arisen simultaneously through this same set of environmental factors. Against this supposition, however, is to be set the fact that these various characters are not always modified in the same direction throughout the entire range of the species. For example, the desert mice have narrower tail stripes and less pigmentation generally than those of Berkeley, whereas the mean tail length is almost identical in the two races. Of course, it is possible to rejoin that the effects of environmental influences in any given case are probably very complex, and that while one set of conditions might call forth parallel modifications in two different characters, another set of conditions might call forth divergent ones. In making such assumptions, we should, of course, be venturing upon very uncertain ground, but I cannot conceive of an explanation of the curious relations here considered, without some sort of appeal to local (i. e. environmental) factors.

I have already stated that when we throw together certain of the local collections and treat them as a single population, a decided positive correlation appears between two characters (tail length and tail stripe)[8] which were not correlated within the local collections taken separately. This, indeed, is a mathematical necessity when two groups of individuals, differing in the mean values of two characters, are mixed together. The characters in question are inevitably found to be correlated in the mixed population. It might seem, at first, to be equally inevitable that the gradual migration and dispersal of these mice would bring about a similar intermixture, resulting in a measurable correlation between two such characters. That this has not actually resulted is doubtless owing to the slowness of the process of dispersal. If these animals were continually traveling great distances and

[8] This would doubtless be true of certain other pairs of characters, for which my data are not yet complete.

in large numbers, there would, without question, occur such a mingling of types as would suffice to bring about this result. The case would be indistinguishable from that of our combining in the same table the measurements of individuals from different local collections. But it does not seem at all probable that these animals indulge in such extensive migrations within their comparatively brief lives. It would probably take many generations of mice before the descendants of any particular local strain would reach a point only twenty miles distant, and this would be doubly true if any form of geographic or ecologic barrier intervened.

Now it is important to point out that such a gradual intermixture of two local races, even if complete, would not result in bringing about the correlation of two characters which did not previously tend to vary together.[9] For, as will be shown in detail later, there seems to be no tendency in hybridization for these various racial characters to be transmitted together. The 'rubidus' mice from Humboldt County have both a considerably longer tail and considerably wider tail stripe than the 'gambeli' mice from Calistoga. But neither in the F_1 nor the F_2 generation of hybrids do we find any more evidence of correlation between these characters than we find in the pure races, considered separately. The same independence in transmission is probably true in respect to tail stripe and foot pigmentation.

Thus it would seem that the racial complex of characters is permanent only so long as mice of the same 'race' breed together, as happens of necessity in nature. There is no linkage among these characters in heredity, or at least this is true of some of the most distinctive ones.

Such a condition of independent transmission is, of course, the familiar one in Mendelian inheritance, where the various unit factors segregate, for the most part, without relation to one another. But here the resemblance ends. As will shortly be

[9] It would, of course, eliminate all local differences of type, unless the modifying agencies continued to operate at a rate sufficient to outweigh the process of diffusion, an assumption which we must make wherever local differences are encountered.

shown, the racial differences under consideration do not depend upon single Mendelian factors.

As to those cases in which two characters are positively correlated in the individual (e.g., tail and foot), this may be due to some sort of "linkage" in inheritance, but may likewise be due to parallel modification by environmental agencies. For the white mouse I demonstrated many years ago that the length of both tail and foot was to a considerable extent dependent upon the temperature of the atmosphere in which the animals were reared, and in the case of Peromyscus I have more than once pointed out that cage-born animals tend to have both of these parts shortened. Such facts as these point to the possibility that the correlation of these parts in nature may be due to the varying incidence of external modifying agencies of some sort.

I shall now pass to the second phase of these studies to be reported upon in the present paper—that, namely, which concerns itself with the crossing of different geographic races. It is significant that the word 'genetics' has, to an increasing extent, come to mean the experimental study of Mendelian unit factors—real or alleged—as revealed by hybridization.[10] And indeed to genetics, in this unwarrantably restricted sense of the word, we are ourselves giving considerable attention in our work with Peromyscus. We have followed the inheritance of several different color mutations, and obtained fairly typical monohybrid and dihybrid ratios, along with some cases which still puzzle us. Some of these results I have already published in preliminary form, and a further report will probably be made during the next few months by Mr. Collins and myself.

My only quarrel is with the contention that all inheritance is Mendelian, whether it seems so or not, and with the endless creation of hypothetical 'unit factors,' to explain every departure from the expected manner of transmission. In this latter class I include the so-called 'multiple factor' explanation of blended inheritance and of the modification of characters through selection.

[10] I recall finding this meaning explicitly given to the word in a recent work.

As is well known, the exponents of this latter hypothesis lay stress upon those undoubted cases in which the second hybrid generation, while showing an intermediate condition, like the first, nevertheless displays a higher variability than the latter. The necessity for such an increase in variability, as a result of segregation, is obvious where a single pair of unit factors is concerned, as in ordinary Mendelian inheritance. That it would be equally necessary if a given character difference were conditioned by two or more pairs of independently segregating factors may readily be proved.[11]

Such a general increase in variability, in later hybrid generations, was, it is worth while noting, well recognized by the early hybridists before the work of Mendel became known. To one who is not committed to the doctrine of the immutability of the 'gene,' such an increased variability is intelligible upon the assumption of a tendency for the parental contributions to segregate from one another during the formation of the germ cells. This tendency may be completely realized, as in the case of typical Mendelian inheritance. It may be overcome, wholly or partially, by a tendency toward fusion, in those numerous cases in which we have a permanent blending of characters, whether or not an increased variability is shown in later hybrid generations.

As between these two theories, the case is by no means as definitely closed as the confident assertions of various recent Mendelian writers would lead one to suppose. Both are still legitimate scientific hypotheses.[12] I personally lean toward the view which seems to me to involve the fewest unproved assumptions—the view, namely, that characters, genetic as well as somatic, may and do actually blend with one another permanently.

I have thus far reared F_1 and F_2 generations from three different crosses among my various races of mice. I am able to

[11] This point has been treated satisfactorily in various recent works (e.g., Babcock and Clausen's 'Genetics,' pages 183–186) and need not receive further consideration here.

[12] This I feel warranted in asserting, despite Castle's recent defection from the ranks of those who uphold the view-point here advocated.

report in the present paper upon unpublished data derived from two of these crosses. The series included here comprise, in one case 97 F_1 and 87 F_2 animals, in the other case, 154 F_1 and 84 F_2 animals. Two further series representing the widest of these crosses still remain to be studied, but the F_2 generations are not yet old enough to kill and measure.

The mice here considered are much freer from abnormalities due to captivity than were the hybrids upon which I have reported in previous papers. I feel, moreover, that I am more nearly in a position to make proper allowance for such abnormalities, and to know when they do and when they do not affect the validity of the results. Reference will be made below to this aspect of the case.

So far as the hybrid series are concerned, I shall restrict myself, in this preliminary discussion, to a comparison of the variability of the F_1 and F_2 generations, in respect to certain characters. In table 4 the standard deviations for five of these characters have been given. These are tail length (relative), foot length, ear length, width of tail stripe, and depth of foot pigmentation. Since two different crosses are under consideration, and the two sexes have been treated separately, there are twenty pairs of figures to be compared, in our endeavor to ascertain the relative variability of the two hybrid generations. Owing to the fact that the absolute measurements for foot length and ear length are closely correlated with those for body length, and since the variability of the various series differs considerably in respect to body length, I have computed the net variability for foot and ear length (see below).

To sum up the outcome of these computations, out of twenty pairs of comparable figures, that for the F_2 generation is greater in 8 cases and less in 4 cases, while the two are equal in 8 others. (I have considered two figures as equal when the difference between them is less than the probable error of that difference.)

It is not, however, certain that the parent races in either of these crosses differ significantly in respect to ear length, while in one of the crosses (Carlotta-Calistoga) it is also questionable whether the wild stocks differ significantly in respect to foot

pigmentation. Considering then, the fourteen remaining figures, representing characters in respect to which the parent races differ unmistakably, we have—

F_2 greater than F_1 in 8 cases
F_2 less than F_1 in 2 cases
F_2 equal to F_1 in 4 cases.

Let us add that in two of the instances in the first of these groups, the differences are scarcely larger than their probable errors, while in only a few of the entire fourteen do they attain anything approaching statistical certainty.

I think it is plain, therefore, that the exponents of the 'multiple factor' hypothesis will derive rather cold comfort from the figures which I have to offer, even though the table as a whole may show a slightly preponderant increase of variability in the second hybrid generation. Of course, one answer is obvious. For studies such as these I have not chosen 'favorable' material. In these wild races, it may be contended, the number of factor mutations for each character has been so great that segregation cannot be expected to manifest itself appreciably in these small series. Such arguments are as unanswerable as they are unconvincing.

Experience warns me that another objection is likely to be made to the validity of these results, although it is an objection which I believe to be utterly irrelevant when brought in this connection. It will be pointed out that each of the parent races with which I am dealing is in reality not pure, but is a mixture of genetically distinct strains. As evidence of this will be cited the wide range of variability within each race, and the fact that, for two characters at least, I have shown these variations to be hereditary. Even if all this is granted, however, and the contentions of the pure-line school be admitted in full, it still seems to me inevitable that we should, on Mendelian principles, have an increased variability in the F_2 generation of hybrids between two such mixed races. For there would in general be more factorial differences between representatives of two geographical races than between two individuals of the same race. The F_1 generation from such a cross would present more heterozygosis

than either parent race, taken by itself, and the chances of the segregation of extreme types would be much better in the F_2 than in the F_1 series. All this seems so obvious that it is difficult to understand how such an objection could be raised seriously.

Passing to a very interesting incidental result of these studies, certain striking differences have been observed between the sexes. The most certain of these relate to the feet and the pelvic bones. When mice of the same size are compared, it is found in all but one of the eight local collections that the average foot length of the males is greater than that of the females. In most cases this difference is statistically significant, whereas in the single exceptional case there is a practical equality between the figures. Also, in all of the four races whose skeletons have thus far been measured, the average length of the innominate bone (pelvis) is greater in the females, this difference, in three of the cases, being large in proportion to its probable error.

It is of interest to note that the two differences just mentioned are of opposite sign. We may profitably consider the bearing of these relations upon certain prevalent ideas regarding the origin of secondary sexual characters. Although many theories have been put forth in this field, it is my understanding that secondary sexual differences, in the higher vertebrates at least, are now commonly supposed to be due largely to the action of internal secretions or 'hormones,' produced by the gonads or by certain cells of these. To simplify the situation, we might assume, in each sex, the existence of a single characteristic hormone which determined all of the secondary sexual characters. In the present instance. the male hormone would, among other things, stimulate the growth of the feet and inhibit that of the pelvic bones. The female hormone, on the contrary, might be supposed to stimulate the growth of the pelvic bones and inhibit the growth of the feet.

Now, according to the foregoing hypothesis, such a state of affairs would inevitably bring about in each sex a negative correlation between feet and pelvic bones. For in each sex there would surely be wide individual differences in the amount of

the hormone formed. As a consequence, we should have more and less masculine males, as well as more and less feminine females. This last, of course, is a quite obvious fact in many species, including man. Granting these differences in the potency of an agent, assumed to modify two characters in opposite directions, a negative correlation between these last would necessarily result when the animals of either sex were treated statistically.

The interesting fact to be reported here is that precisely the opposite relation is found to obtain. Foot length and the length of the pelvis are found to be positively correlated, with a high degree of probability.[13] Accordingly, if these two secondary sexual differences are conditioned by 'hormones' at all (which does not, in itself, seem improbable), there must be at least two such hormones, which vary independently of one another.

It may be permissible to call attention to the similarity between this situation as regards the sexes and a condition already discussed in considering our geographic races. The case was mentioned of two characters (indeed, these same two characters) which were correlated within the single race, but which nevertheless were found to have varied in opposite directions, when certain races were compared with one another. Whether such an agreement in behavior in these two cases has any general significance I cannot even conjecture.

CONSIDERATION OF DATA IN DETAIL

The ensuing section will consist, for the most part, of a discussion of some figures and tables which present certain portions of my data in graphic or in summarized form. It is upon these that the generalized statements in the first section were chiefly based.

[13] I will mention here that the evidence for the reality of such a correlation is somewhat stronger than the figures comprized in table 3. (See discussion below.) Measurement of the bones of four more races, now available to me, should render this point decisive.

The map of California (fig. 1) shows the position of the eight stations at which my most extensive collections have been made. Two of these stations (La Jolla and Berkeley) were chosen largely as matters of convenience, but the other points were

Fig. 1 Map of California, showing the eight stations at which representative collections of mice have been trapped.

selected with reference to definite geographical and biological conditions. Mice of the species under consideration (Peromyscus maniculatus) inhabiting four of these stations (Eureka, Carlotta, Fort Bragg, Duncan Mills) have been assigned by

recent systematists to the subspecies rubidus Osgood. Those from Calistoga, Berkeley, and La Jolla are assigned to gambeli (Baird), while ones from Victorville are assigned to the characteristically desert race sonoriensis (Le Conte).

In table 1 we find the mean values given for most of the characters which have been subjected to measurement.[14] It must be explained, however, that the mean values here given have, for most characters, been 'corrected' in such a way as to be comparable with one another. This was rendered necessary by the fact that the mean body length (total length minus tail length) differed considerably among the various collections, owing largely to the inclusion of differing numbers of immature individuals.[15] Since most of the characters here considered are rather strongly correlated with body length, their mean values in these different sets would obviously have not been directly comparable.[16]

The figures (or most of them) have accordingly been corrected for each series in such a way as to give their most probable value had the mean body length of the series in question been 90 mm. This was accomplished, I need hardly say, by the use of the so-called regression coefficient.[17] As a matter of fact, the corrections which were applied were in most cases small in comparison with the differences between the various races. They were largest in the Fort Bragg series, which contained a greater proportion of immature animals than did any of the others.

[14] Weight and skull width have been omitted, for reasons which need not here be discussed. Foot-pigmentation has only recently been included among the characters measured, so that figures are not yet available for these races. Body length is not included for reasons stated in the next paragraph.

[15] All animals below 80 mm. in body length were, however, arbitrarily excluded.

[16] In a more complete presentation of these results, I shall include the original averages, but this does not seem necessary for the present.

[17] The correction is obtained by the equation $x = r \frac{\sigma_x}{\sigma_y} y$, in which x represents the difference sought between the corrected and obtained values for a given character, r the correlation between the character in question and body length, σ and σ_y the standard deviations for this character and for body length, respectively, and y the difference between the standard value (90 mm.) and the actual mean body length of the lot in question. Of course, all these standard deviations and correlation coefficients had to be first computed for each race and sex separately.

TABLE 1[1]

Mean values of certain characters in eight local 'races,' reduced to standard body length of 90 mm.

		NUMBER	TAIL ABSOLUTE	TAIL PER CENT	FOOT	EAR	TAIL STRIPE	VERTEBRAE	PELVIS	FEMUR	SKULL LENGTH
Eureka.....	♂	87	93.70 ±0.42	104.45±0.38	21.48 ±0.04	17.56 ±0.06	42.51 ±0.45	28.03 ±0.09	17.42 ±0.07	15.93 ±0.06	25.71 ±0.05
	♀	59	93.31 ±0.55	103.37±0.52	21.06 ±0.05	17.70 ±0.07	41.96 ±0.53	28.37 ±0.11	17.65 ±0.11	15.80 ±0.08	25.67 ±0.06
Carlotta....	♂	63	93.54 ±0.46	104.64±0.42	21.52 ±0.06	17.33 ±0.07	40.92 ±0.44				
	♀	53	93.90 ±0.62	103.78±0.66	21.08 ±0.07	17.14 ±0.09	42.29 ±0.52				
Fort Bragg.	♂	59	92.65 ±0.51	100.70±0.48	21.53 ±0.05	16.95 ±0.07	39.32 ±0.46				
	♀	41	90.58 ±0.66	100.07±0.52	20.96 ±0.06	17.08 ±0.10	40.34 ±0.43				
Duncan Mills.....	♂	51	84.98 ±0.51	94.69±0.49	20.81 ±0.07	16.73 ±0.08	38.60 ±0.56				
	♀	40	82.67 ±0.68	91.51±0.52	20.50 ±0.07	16.54 ±0.09	37.45 ±0.45				
Calistoga...	♂	67	76.81 ±0.45	85.63±0.44	20.15 ±0.05	16.75 ±0.06	33.86 ±0.44				
	♀	54	77.11 ±0.52	85.60±0.47	19.76 ±0.06	16.96 ±0.08	35.08 ±0.55				
Berkeley...	♂	55	72.93 ±0.41	81.80±0.39	19.87 ±0.05	16.10 ±0.06	35.31 ±0.51	25.85 ±0.10	17.56 ±0.09	15.72 ±0.08	24.67 ±0.07
	♀	34	73.38 ±0.66	81.53±0.47	19.63 ±0.07	16.40 ±0.07	36.85 ±0.55	25.87 ±0.10	18.19 ±0.14	15.71 ±0.11	24.64 ±0.10
La Jolla....	♂	105	75.79 ±0.36	84.36±0.35	20.02 ±0.06	17.72 ±0.05	32.08 ±0.33	26.45 ±0.07	17.56 ±0.06	15.87 ±0.06	25.04 ±0.05
	♀	70	75.26 ±0.40	83.44±0.35	20.13 ±0.04	17.88 ±0.08	32.61 ±0.41	26.38 ±0.09	18.17 ±0.09	15.79 ±0.09	24.85 ±0.08
Victorville..	♂	78	72.90 ±0.43	81.29±0.44	19.97 ±0.04	17.22 ±0.06	27.49 ±0.32	25.93 ±0.11	17.33 ±0.06	15.87 ±0.06	24.94 ±0.06
	♀	62	73.28 ±0.42	81.30±0.45	19.56 ±0.06	17.30 ±0.06	28.92 ±0.36	26.43 ±0.09	18.03 ±0.10	16.03 ±0.08	24.84 ±0.06

[1] Certain of these figures will be found to be slightly different from those contained in my 1918 paper, owing to the inclusion here of larger numbers of mice in the Berkeley and La Jolla series.

For relative tail length, relative width of the tail stripe, and number of vertebrae no corrections have been introduced, since the first of these characters is correlated feebly and the last two probably not all with body length.

Another way of comparing the mean values of these various characters in series of animals which differ in size has been employed by me in earlier papers. This is to divide each series into groups containing individuals of approximately the same size, and to compare the means of the corresponding size groups of different series. The mean difference between the two series under comparison may readily be computed according to a simple formula.[18] Such mean differences I have not yet calculated, however, for the present material.

Figure 2 is based upon the corrected averages referred to above, the mean of the figures for the two sexes (not weighted) being employed for each race. The differences between these various racial means are plotted to scale along the vertical lines. The bone measurements have thus far been taken for only four of the eight races, though the various bones have already been prepared for measurement.

It will be noted that for no two of the characters considered is the arrangement exactly the same. The nearest approach to agreement is found between the scales for tail and foot length, the chief difference being that for the latter character there are fewer distinguishable grades. The order for tail stripe is the same as that for tail length with this important exception, that Berkeley has been transferred from near the bottom of the scale to a point above the middle.

Skull length in the four races for which figures are available follows nearly the same order as tail and foot length.

In respect to the number of caudal vertebrae, Eureka stands at the top of the scale, as was true for all of the four characters just considered. The differences among the other four stations are, however, of doubtful significance in the case of the vertebrae.

[18] Journal of Experimental Zoölogy, April, 1915, p. 346.

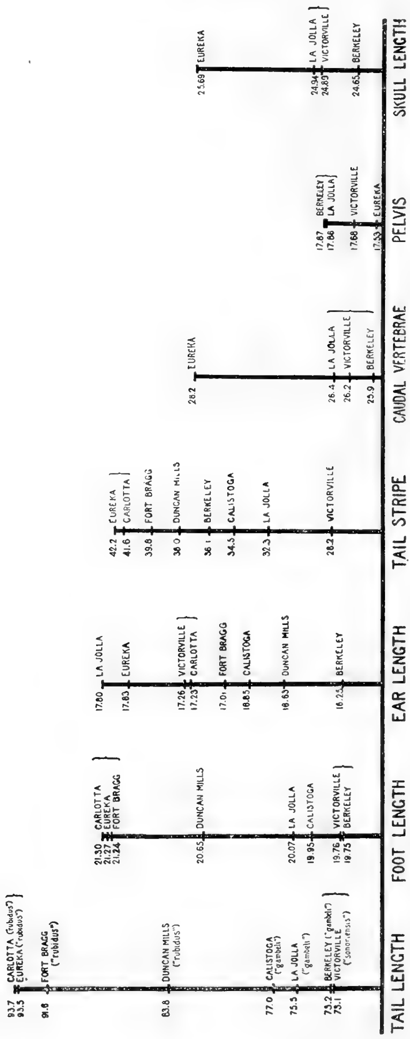

Fig. 2 Diagrams showing gradation of the local 'races,' in respect to the mean values of seven different characters. The means have all been reduced to a common basis, as explained in the text. Skeletal measurements have thus far been made for only four of the local collections.

As to the two remaining characters (length of the ear and of the pelvis) the arrangement of the stations follows a quite different order in the two cases, while for neither is the order like that for any of the characters previously considered. It must be said, however, that ear length is a rather erratic character in its behavior, some rather perplexing and contradictory results having been obtained in the course of these studies. The outstanding fact here is that the two extremes for ear length are displayed by the Berkeley and the La Jolla animals, both of which 'races' are commonly assigned to the single subspecies gambeli. These differ in average ear length by about $1\frac{3}{4}$ mm., the difference being undoubtedly a real one, characteristic of the mice of the two localities. As to the innominate bone, the difference between the two extremes here shown is doubtless statistically significant, despite its small magnitude. The lesser differences are not so certain.

Two other modes of portraying these racial differences are shown in figures 3 and 4 and in figure 5, respectively. The former type of chart has been employed by me in many previous papers dealing with variation and heredity in mice. In the case of the present material, I have plotted graphs of this sort for most of the characters here discussed, though only one of these (that for foot length) has been included in this paper. As I have frequently had occasion to explain previously, each of the 'curves' in this figure results from connecting the mean values of this character for the various size-groups into which the individuals of a given race have been divided. Thus, animals of the same size are represented by corresponding points on the various 'curves,' and the comparisons between the races are strictly legitimate. The essential agreement between figures 3 and 4 and the scale for foot length in figure 2 is obvious.

Figure 5 consists of histograms based upon the individual frequencies of the various values for two characters in the eight local collections. These, of course, have the advantage of showing the total range and variability of the respective characters within each race. Such a method of representation would be inappropriate for most of the other characters under discussion,

since the variability of these is so largely dependent upon the variability in the size of the animals concerned.

As has already been noted, the order of arrangement is much the same for the two characters portrayed. The only difference concerns the relative positions of the three 'gambeli' races.

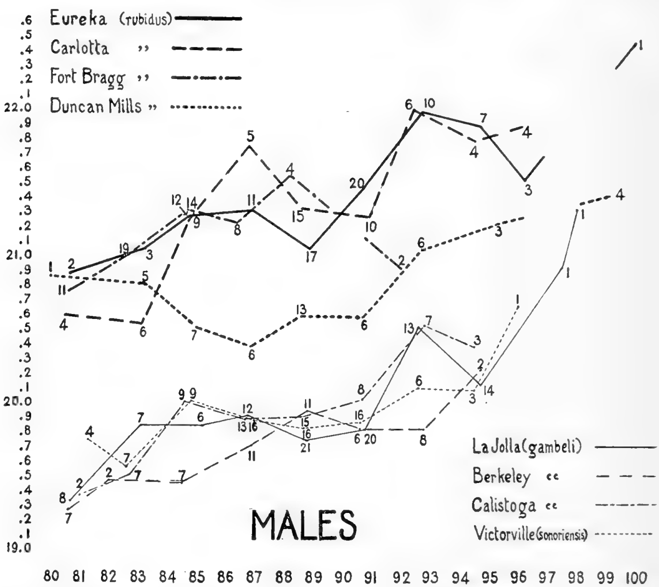

Fig. 3 Graphs showing foot length in the eight races (males). Body length is represented on the horizontal axis, foot length on the vertical one. The figures along the various 'curves' indicate the number of individuals in the respective size groups. Each 'curve' connects the group-means for a single local 'race.'

These three, however, taken together, occupy in both cases the same position, relative to the other races. The broad overlapping of adjacent races and the large degree of distinctness of the extremes are well shown in this figure.

Passing to a consideration of the correlations among the various characters, it will be seen from table 2 that all of the

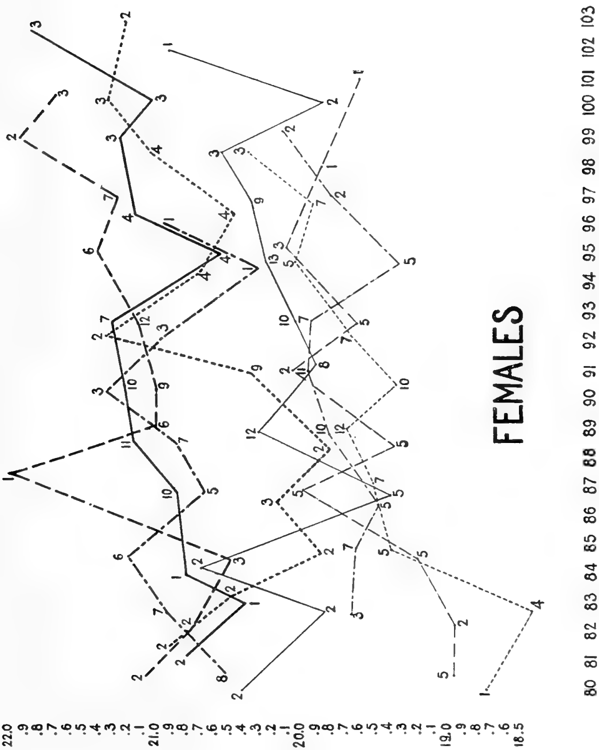

Fig. 4 Foot length of females (see fig. 3).

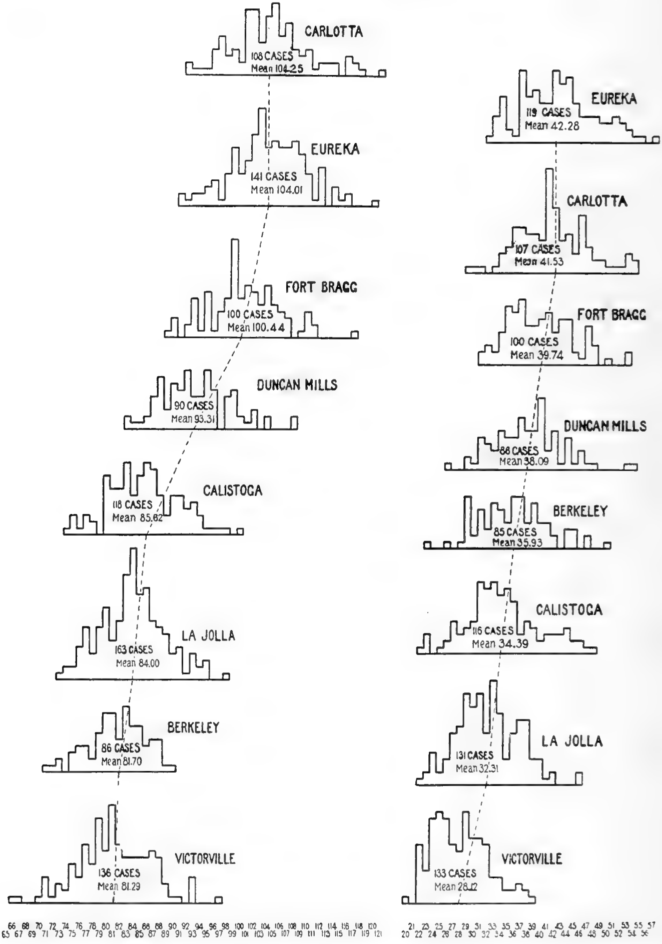

Fig. 5 Histograms showing distribution frequencies for the various values of relative tail length (percentage of body length) and relative width of the tail stripe (percentage of circumference) in the eight local collections (sexes combined). The broken lines connect the means of the various series.

TABLE 2[1]

Correlations between body length and other characters

		NUMBER	BODY AND TAIL (ABSOLUTE)	BODY AND FOOT	BODY AND EAR	BODY AND PELVIS	BODY AND FEMUR	BODY AND SKULL	BODY AND TAIL PER CENT	BODY AND TAIL STRIPE
Eureka	♂	87	+0.58	+0.44	+0.31	+0.78	+0.72	+0.68	−0.05 ±0.07	+0.09 ±0.08
	♀	59	+0.59	+0.23	+0.12	+0.74	+0.72	+0.58	−0.33 ±0.08	−0.03 ±0.09
Carlotta	♂	63	+0.61	+0.50	+0.52				−0.26 ±0.08	−0.06 ±0.09
	♀	53	+0.44	+0.28	+0.58				−0.34 ±0.09	+0.10 ±0.10
Fort Bragg	♂	59	+0.63	+0.30	+0.19				+0.20 ±0.08	−0.05 ±0.09
	♀	41	+0.74	+0.18	+0.38				+0.12 ±0.10	0.00 ±0.11
Duncan Mills	♂	51	+0.57	+0.31	+0.28				−0.24 ±0.09	+0.10 ±0.10
	♀	40	+0.73	+0.45	+0.51				−0.21 ±0.10	+0.30 ±0.10
Calistoga	♂	67	+0.48	+0.40	+0.33				−0.07 ±0.08	0.00 ±0.08
	♀	54	+0.57	+0.09	+0.41				−0.16 ±0.09	+0.01 ±0.09
Berkeley	♂	55	+0.53	+0.40	+0.29	+0.84	+0.88	+0.81	−0.25 ±0.09	+0.03 ±0.09
	♀	34	+0.82	+0.44	+0.62	+0.91	+0.87	+0.82	+0.03 ±0.12	−0.09 ±0.12
La Jolla	♂	105	+0.49	+0.27	+0.52	+0.82	+0.81	+0.83	−0.16 ±0.07	−0.11 ±0.07
	♀	70	+0.63	+0.14	+0.57	+0.87	+0.85	+0.88	−0.25 ±0.08	+0.25 ±0.08
Victorville	♂	78	+0.44	+0.18	+0.38	+0.77	+0.76	+0.78	−0.10 ±0.08	−0.26 ±0.07
	♀	62	+0.41	+0.51	+0.38	+0.81	+0.84	+0.67	−0.34 ±0.08	+0.17 ±0.09
Mean		978	+0.561	+0.317	+0.397	+0.812	+0.800	+0.760	−0.153	+0.018

[1] The number of individuals upon which a given correlation is based is frequently somewhat smaller than the number stated in the table, owing to lack of certain measurements in damaged specimens. Apparent discrepancies in the probable errors are thus accounted for.

absolute values which have been determined are strongly and positively correlated with body length.[19] The correlation is highest in respect to the three skeletal characters which are included. Of the two relative characters, the ratio of tail to body is seen to be negatively correlated with body length. That is, larger mice have proportionally slightly shorter tails. On the other hand, the relative width of the tail stripe (ratio to circumference of tail) does not appear to be correlated significantly with the general size of the animal.

Much more instructive from our point of view are the correlations of the various characters, other than body length, with one another (table 3). That between tail stripe and relative tail length has been computed in the entire undivided populations, irrespective of size. Of the sixteen different figures (the races and sexes being treated separately), it will be seen that six are positive and ten negative, the mean for the entire series being slightly negative. Since, however, the probable errors for these single coefficients are, for the most part, nearly or quite as great as the coefficient themselves, it is quite unlikely that the preponderance of the negative values is significant. We may fairly assume, therefore, that the two characters are not appreciably correlated.

I have likewise thrown together (though not in the present table), the data from the four 'races' taken north of San Francisco Bay, and treated the entire lot as a single population, the sexes, however, being dealt with separately. Deviations from the grand averages were employed in the computations. The stations represented are Calistoga, Duncan Mills, Fort Bragg, and Carlotta.[20] Fairly high positive coefficients now appear between the two characters last mentioned (+0.366 for the 230 males and +0.351 for the 176 females). The significance of this fact has been discussed in the preliminary section of the present paper.

[19] Exception should be made of the number of caudal vertebrae.

[20] Since the Carlotta and Eureka collections are nearly identical in their mean characters, I have not included the latter. The four sets used were trapped and measured during the same season and are possibly more nearly comparable, on this account, than ones taken in different years.

Two characters which are correlated with a high degree of certainty are relative tail length and the number of caudal vertebrae. It seems, on first thought, curious that relatively longer tails should tend to have a larger number of vertebrae, while the absolute length of this appendage should play no appreciable part in the matter. I have not tested directly the correlation between absolute tail length and the number of caudal vertebrae, but the fact that the longer animals of my series (having, as a consequence, longer tails) do not have more vertebrae than the shorter ones, renders improbable the existence of such a correlation. It must be pointed out here that the slight differences met with in the number of the caudal vertebrae have little part in determining the differences in tail length, whether between races or individuals. These depend mainly upon the size, rather than the number of the separate bones.

In determining correlations between the various other pairs of characters, a different procedure has been adopted, owing to the fact that these characters are all strongly correlated with body length, and therefore, in a population of mixed size, necessarily are correlated with one another. For this, as well as for other purposes, I have divided up the animals of each local collection into groups of individuals differing by less than 2 mm. Correlations have been determined for each size-group containing ten or more individuals, and the means of these coefficients employed.[21]

Positive coefficients of probable significance have been obtained for tail and foot and for tail and skull. The correlation between tail and ear length is far less certain, while none appears to exist between the tail and the pelvis. On the other hand, the foot and the pelvis seem to be correlated with a considerable degree of probability. It is worth adding that the existence of a positive correlation between tail length and foot length, as well as between foot length and that of the pelvis, is made yet more probable from an inspection of certain series of mice which were measured before my methods were fully standardized, and which have therefore not been included in the present computations.

[21] The various methods here used have been discussed in a previous paper (Journal of Experimental Zoölogy, April, 1915).

TABLE 3

Correlations between various characters. In all except the first two columns, the figures represent correlations among individuals of the same body length (see text)

		Tail per cent and tail stripe	Number	Tail per cent and vertebrae	Number	Tail and foot	Number	Tail and ear	Number	Tail and pelvis	Number	Tail and skull	Number	Foot and pelvis	Number
Eureka	♂	−0.07 ±0.08	69	+0.31	72	+0.08	60	+0.02	58	−0.08	57	+0.19	58	+0.16	66
	♀	−0.08 ±0.10	49	+0.33	48	+0.25	30	+0.22	20	−0.16	30	+0.13	30	+0.06	31
Carlotta	♂	+0.14 ±0.09	58			+0.23	24	+0.14	24						
	♀	−0.13 ±0.10	47			+0.32	12	+0.32	12						
Fort Bragg	♂	−0.14 ±0.09	59			−0.05	44	−0.14	45						
	♀	−0.01 ±0.11	41												
Duncan Mills	♂	−0.01 ±0.10	48			−0.39	13	−0.09	13						
	♀	−0.17 ±0.10	38												
Calistoga	♂	+0.06 ±0.08	65			+0.42	29	−0.01	28						
	♀	−0.05 ±0.09	50			+0.28	21	+0.28	20						
Berkeley	♂	+0.17 ±0.09	51	+0.45	52	+0.31	22	+0.34	22	−0.28	22	+0.06	22	−0.08	22
	♀	−0.16 ±0.12	32	+0.34	30										
La Jolla	♂	+0.01 ±0.07	83	+0.38	89	+0.38	76	+0.24	78	+0.20	76	+0.08	78	+0.36	78
	♀	+0.10 ±0.09	61	+0.13	61	+0.14	33	+0.06	23	+0.11	33	+0.02	23	+0.18	35
Victorville	♂	+0.09 ±0.08	72	+0.23	58	+0.34	45	−0.11	44	+0.18	45	+0.32	44	+0.19	45
	♀	−0.17 ±0.08	59	+0.35	56	+0.12	22	+0.65	23	−0.48	23	+0.01	23	+0.34	22
Mean		−0.016	882	+0.314	466	+0.210	431	+0.118	410	+0.001	286	+0.134	278	+0.204	299

Passing now to the hybridization of races, I shall not discuss this at any great length in the present paper, owing to the fact that two other important series remain to be measured, and I plan to publish a more complete account when these additional data are available. I have not, therefore, included a table giving the mean values of the various characters in the different crosses. Regarding these mean values, I may say that, in respect to characters in which the parent races differ, the figures for both the F_1 and F_2 generations, are intermediate, though not always midway between those of the parents. And with one exception (foot length in the Eureka-Victorville cross), the mean values agree pretty closely in the two hybrid generations. In this exceptional instance there is a perceptible reduction in the mean foot length in the second generation.

It is important to know to what degree these hybrids have been modified by captivity, and particularly whether any of the relations to be discussed below are attributable to this cause. In addition to frequent sterility and a greater tendency toward adiposity, the chief modifications to be observed in many of the cage-bred mice consist in: 1) reduction in general body size; 2) reduction in the relative length of the appendages and in the width of the tail stripe, and, 3), for some characters, at least, an increase in variability.

Now, in both of the crosses to be considered, the mean body length is somewhat less in the F_2 generation than in the F_1, and in one of these (Eureka-Victorville) there has occurred a small reduction, both relative and absolute, in the mean length of the foot. There seems to be evidence, therefore, that the F_2 generation is somewhat less normal than the F_1. So far as this fact has any bearing on the comparative variability of the two hybrid generations, it must be stated that the probable effect of an increase of abnormality would be an increase of variability. As regards tail length, at least, I have definite evidence of this in comparing the standard deviations of wild and cage-bred mice.[22] The figures for the latter are much larger. Thus, if abnormality

[22] I have not yet computed standard deviations for most of the characters in cage-bred animals of the pure races.

due to captivity be really a factor in accounting for the relations to be discussed presently, its mode of operation has been to increase rather than to decrease the appearance of segregation.

It should be said, too, that the F_2 generation, in the Carlotta-Calistoga cross, was visibly more normal than in the Eureka-Victorville one. Indeed, in the former, save for the fact that most of the animals were appreciably smaller than wild ones, there was rarely any indication of abnormality in either hybrid generation. It is in this set, let us note, that we have the least indication of segregation.

The possible bearing of this disturbing element upon my hybridization data will be discussed more fully in a later paper, in which I shall present the results of a more satisfactory series of sonoriensis-rubidus hybrids. For the present, let me repeat that I believe the possibility of such an influence merely serves to weaken the slight evidence which I have found in favor of gametic segregation in these racial crosses.

Let us then consider the comparative variability of the two hybrid generations. Figures 6 and 7 show the actual frequency distributions of the two crosses here considered for two characters which admit of very precise measurement and which are largely independent of the size of the individual. While exact quantitative comparisons between such figures are out of question, they certainly afford little evidence of an increase of variability in the second hybrid generation, as compared with the first hybrid generation or with either parent race.

A more accurate comparison of variability among these different lots is afforded by table 4, which presents the standard deviations for the parent races and the two generations of hybrids. So far as tail-to-body ratio, foot lengths, and ear lengths are concerned, these standard deviations have been subjected to a 'correction,' as were the averages in table 1. This is for the reason that these three characters, particularly the two last, are correlated with the general size of the animals. Part of their gross variability, therefore, may be supposed to have resulted from the variability of their respective series as regards body length. I have accordingly computed their net variability in

each case, by multiplying the original standard deviation by the factor $\sqrt{1-r^2}$, in which r is the correlation between body length and the character in question.[23]

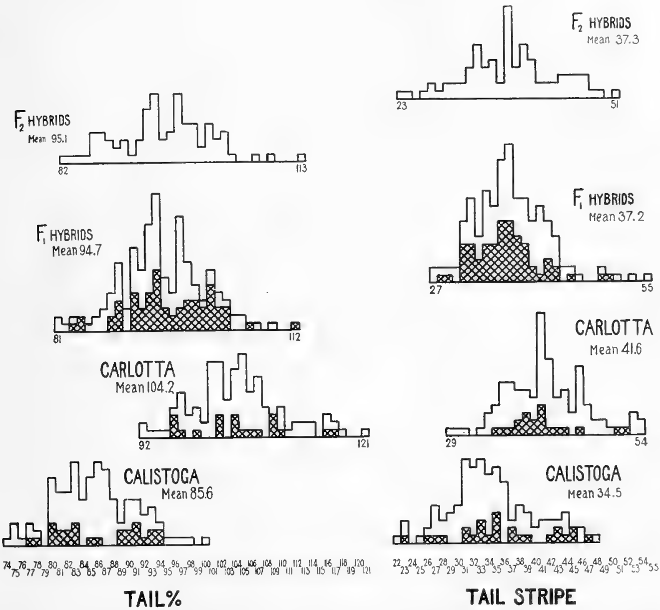

Fig. 6 Histograms permitting comparison of variability of the parent races and the F_1 and F_2 generations of hybrids, with respect to relative tail length and tail stripe, in the Carlotta-Calistoga cross (sexes combined). Shaded squares, in pure races, indicate parents of F_1 broods. Shaded squares in F_1 indicate parents of F_2 broods and sibs of same.

A brief analysis of these figures has been made in the introductory section of this paper. No further discussion of them seems worth while at the present time.

[23] This gives the same result as if each single deviation for the character in question were corrected by making allowance for its correlation with body length, as was done for the mean values in table 1. For this and certain other information of a mathematical nature I am indebted to my colleague, Dr. G. F. McEwen.

Reference has also been made in the introduction to the probability that the various characters which are associated together in a single local race do not tend to cohere in hybridization, but that they appear to vary nearly or quite as independently of one another in hybrids as they do in pure races. The rather meagre data thus far available for a test of this question are somewhat

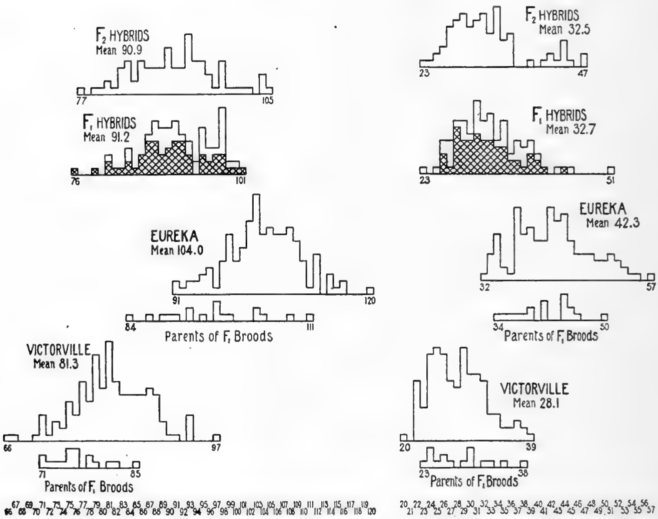

Fig. 7 Variability in Eureka-Victorville cross (see legend for figure 6). The actual parents of the F_1 animals here belonged to a cage-born series, and are therefore not included in the larger polygons for the parent races, which are based upon wild material exclusively.

contradictory. But they do not, I think, furnish any support to the view that racial characters tend to segregate together, if indeed any segregation, in a Mendelian sense, occurs here at all.

I will first consider the question of the correlation of relative tail length and width of tail stripe, characters which have been shown to lack significant correlation within the single races, though they are plainly associated with one another when cer-

TABLE 4

Standard deviations of parent races and of two hybrid generations. Figures so corrected as to give net variability (see text)

Cross	Sex		NUMBER	TAIL: BODY	FOOT	EAR	TAIL STRIPE	FOOT PIGMENTATION
Carlotta-Calistoga	♂	Calistoga, P...	67	5.33 ±0.32	0.571 ±0.033	0.733 ±0.043	5.34 ±0.31	
		Carlotta, P....	63	4.68 ±0.29	0.636 ±0.038	0.685 ±0.041	4.99 ±0.31	
		Hybrids, F_1...	81	4.98 ±0.27	0.658 ±0.035	0.822 ±0.044	5.44 ±0.29	0.84 ±0.04
		Hybrids, F_2...	41	5.40 ±0.40	0.550 ±0.041	0.772 ±0.058	5.69 ±0.43	0.75 ±0.06
	♀	Calistoga, P...	54	5.03 ±0.33	0.604 ±0.040	0.783 ±0.051	5.75 ±0.39	
		Carlotta, P....	53	6.43 ±0.44	0.748 ±0.049	0.774 ±0.051	5.28 ±0.36	
		Hybrids, F_1...	73	6.17 ±0.35	0.639 ±0.036	0.942 ±0.053	4.10 ±0.23	0.88 ±0.05
		Hybrids, F_2...	43	5.99 ±0.43	0.618 ±0.045	0.750 ±0.055	6.10 ±0.45	0.81 ±0.06
Eureka-Victorville[1]	♂	Eureka, P.....	87	5.09 ±0.26	0.533 ±0.027	0.791 ±0.042	5.52 ±0.32	
		Victorville, P..	78	5.70 ±0.31	0.557 ±0.031	0.704 ±0.039	4.02 ±0.22	
		Hybrids, F_1...	51	4.35 ±0.29	0.570 ±0.038	0.800 ±0.053	4.64 ±0.31	0.82 ±0.05
		Hybrids, F_2...	50	5.48 ±0.38	0.690 ±0.047	0.801 ±0.055	3.57 ±0.25	0.92 ±0.06
	♀	Eureka, P.....	59	5.48 ±0.35	0.560 ±0.034	0.736 ±0.046	5.53 ±0.37	
		Victorville, P..	62	4.89 ±0.30	0.569 ±0.035	0.606 ±0.037	4.08 ±0.25	
		Hybrids, F_1...	46	4.41 ±0.32	0.617 ±0.043	0.658 ±0.046	5.04 ±0.36	0.87 ±0.06
		Hybrids, F_2...	37	5.87 ±0.46	0.846 ±0.066	0.720 ±0.057	6.57 ±0.52	1.01 ±0.08

[1] For the parent races, in this cross, the figures for the wild mice have been employed, although cage-bred animals were actually used in this case. I will not here take time to justify this procedure.

tain of these races are compared. Taking the four coefficients for our two crosses, in the F_1 generation (males and females being dealt with separately), we find two positive and two negative figures, the mean being +0.036, which can hardly be regarded as significant under the circumstances. The condition of the F_2 lots is doubtless a fairer test of this question. Of the four figures for this generation, three are positive and one negative. the mean of the four being +0.105. That the latter fact can hardly be regarded as furnishing evidence for an actual positive correlation between these characters is evident when we consider that in the Carlotta-Calistoga cross the mean figure for the two sexes is negative, this negative condition being outweighed by a positive one in the Eureka-Victorville series. It does not seem likely, that the two crosses would be found to differ in this regard if adequate material were available.

Another pair of characters for which I have computed correlation coefficients in these series of hybrids are tail stripe and foot pigmentation.[24] Of the eight coefficients here computed, four (the Eureka-Victorville ones) were positive, the other four (the Carlotta-Calistoga ones) being negative. In this case, however the negative figures outweigh the positive ones, both in the F_1 and the F_2 series, the means for the two generations being −0.184 and −0.035, respectively. It must be pointed out, too, that the most nearly 'significant' figures, taken singly, are those of the Carlotta-Calistoga cross, whereas it is doubtful whether these two races differ appreciably in their mean foot pigmentation. This fact renders it probable that all of these coefficients are 'accidental;' that is to say, that they have no bearing upon the special problem under discussion.

[24] The pigmentation of the sole of the (left) foot has been rated according to an arbitrary scale of five grades. The feet of the mice were preserved in 70 per cent alcohol and later transferred to glycerin.

Resumen por el autor, C. M. Child.
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Estudios sobre la dinámica de la morfogénesis y la herencia en la reproducción experimental. X. Frecuencia de la cabeza de Planaria dorotocephala en relación con la edad, nutrición y actividad motríz.

El autor ha descrito en trabajos anteriores el hecho de que el nuevo extremo anterior producido durante el desarrollo regulador de trozos aislados de Planaria dorotocephala varía desde el estado de cabeza normal, pasando por varios grados de inhibición en el desarrollo de dicho órgano, hasta producir una acefalia completa. Por razones descriptivas ha distinguido cinco tipos diferentes de extremo anterior: normal, teratoftálmico, teratomórfico, anoftálmico y acéfalo. La palabra "frecuencia de la cabeza" significa la frecuencia con que aparecen estos diferentes tipos en un lote de trozos del animal, bajo condiciones determinadas. La frecuencia de la cabeza es menor en los trozos de animales fisiológicamente mas jóvenes (mas pequeños) que en los de animales fisiológicamente mas viejos (mayores). Lo mismo sucede en los trozos procedentes de animales mal alimentados. Es por el contrario mayor en los trozos estimulados frecuentemente en el sentido locomotor, durante varias horas después de seccionados, que en los trozos que no se estimulan. La misma serie de formas del extremo anterior aparecen bajo condiciones fisiológicas diferentes y bajo condiciones externas físicas y químicas diferentes, consistiendo los cambios producidos experimentalmente en cambios de la frecuencia de las diferentes formas. Este efecto no específico de los factores fisiológicos y externos indica que la acción de ambos grupos de factores es esencialmente cuantitativa. La interpretación cuantitativa de los cambios en la frecuencia de la cabeza, mencionados previamente, es aplicable a los hechos expuestos en el presente trabajo.

Translation by Jose F. Nonidez
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STUDIES ON THE DYNAMICS OF MORPHOGENESIS AND INHERITANCE IN EXPERIMENTAL REPRODUCTION

X. HEAD-FREQUENCY IN PLANARIA DOROTOCEPHALA IN RELATION TO AGE NUTRITION AND MOTOR ACTIVITY

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THREE FIGURES

Analytic investigation of reconstitution or regeneration in isolated pieces of Planaria dorotocephala has shown that the process is by no means uniform in different pieces, even in those from the same animal. The structures produced at the anterior ends of pieces present the most interesting conditions as regards variation, for they constitute a graded series ranging from normal heads at one extreme, through various degrees of incomplete head development, to a completely headless condition in which the wound at the anterior end merely heals and nothing further occurs. In spite of the fact that they actually constitute a graded series, it has been found convenient for purposes of analysis to divide the different types of anterior end into several groups representing different degrees of departure from the normal and approach toward the headless condition. These different groups have been named, defined, and figured in earlier papers (Child, '11 a, '15 b, p. 106, '16). Investigation has shown further that the character of the anterior end developing in an isolated piece varies in a definite way with the length of the piece or the fraction of body length represented by it and with region of body from which it is taken (Child, '11 b, d). Moreover, it has been found possible to control and alter experimentally to a very high degree the character of anterior end produced. The results of experiments with KNC (Child, '16) and temperature (Behre, '18)

have already been published in full, the results of various other experiments including some of those recorded in the present paper have appeared in brief preliminary form (Child, '11 a), and further experiments on the action of various anesthetics are in progress. These experiments have demonstrated that the various types of anterior end produced by the action of external chemical and physical agents are the same as those which arise in relation to length of piece, region of body, and other physiological conditions.

In most of the work along this line five types or categories of anterior end have been distinguished (Child, '11 a, '16): normal, a head with two distinct eye-spots, lateral cephalic lobes, and pointed tip, as in nature; teratophthalmic, a head of normal shape, but with eye spots more or less approximated, to the median line, often unequal in size, or more or less completely fused in consequence of failure of the median region of the cephalic ganglion to develop completely (Child and McKie, '11); teratomorphic, a more extreme degree of inhibition of the median region, showing a single or completely fused eye spots in the median line and cephalic lobes more or less approximated at the front of the head, in consequence of incomplete development of the median region of the tip; anophthalmic, with a distinct outgrowth with rudimentary ganglion, but without eye spots; acephalic or headless, with mere healing of the wound and no outgrowth. The term 'head-frequency' has been used to indicate the frequency with which these different types of anterior end occur in a given lot of pieces.

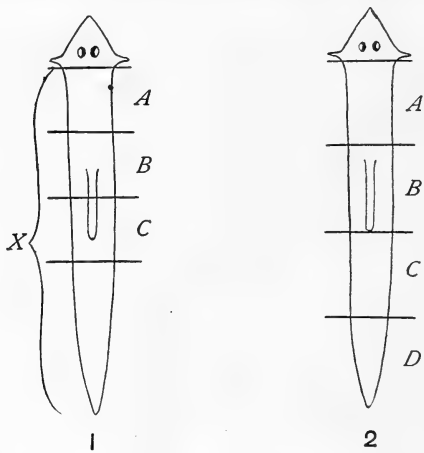
The present paper is concerned with the influence of certain physiological conditions upon head-frequency. The methods of experimental procedure are essentially similar to those described in earlier papers. Each lot of animals used consists of individuals of the same length from a single laboratory stock kept under known conditions of nutrition, temperature, etc., for at least several weeks before the experiment. Each lot of pieces isolated from such animals represents as nearly as possible a given fraction of length and a given region of the body. In all series tabulated except one, the experimental lots consist of fifty pieces each and

all tabulated data are in percentages. It may be pointed out once more that in these head-frequency data only differences greater than 10 per cent are to be regarded as significant. The sources of error are such that it is not safe to attribute definite significance to differences less than this.

HEAD-FREQUENCY IN RELATION TO SIZE OR PHYSIOLOGICAL AGE

It has been shown that susceptibility to lack of oxygen (Child, '19 c), rate of CO_2 production (Child, '19 a), and oxygen consumption (Hyman, '19 a, b, c) all decrease with increasing size in P. dorotocephala, and Allen ('19 a) has recently recorded a similar decrease in oxygen consumption with increasing size in two other species. As regards the question whether size may be regarded as a criterion of physiological age, attention may be called to several points: First, animals sexually produced are approximately equal in size at the time of hatching, therefore the size of such an animal at any given time represents the amount of growth it has undergone, and if physiological senescence is associated with growth and progressive development, the animal must grow old as it becomes larger. In cases where comparison has been possible, animals asexually produced have been found to show approximately the same physiological condition in all respects as sexually produced animals of the same size. Second, the physiological changes which normally accompany growth and progressive development have been prevented, at least so far as could be determined, in Planaria velata by giving only sufficient food to maintain a given size (Child, '14 c). Third, animals of the same size and living under similar conditions are much more alike physiologically than animals of different size. Fourth, sexual maturity occurs only at a relatively advanced physiological stage when a certain size and metabolic condition are attained, and can be prevented from occurring by insufficient feeding or by frequent regeneration. Fifth, the physiological changes with increasing size in Planaria are similar to those usually regarded as characteristic of physiological senescence in the higher animals and man. In short, certain progressive physiological changes

occur with increase in size in Planaria as in other animals generally, and size therefore serves as a criterion of physiological condition so far as it concerns these changes. Whether we call these changes physiological senescence or something else is purely a matter of definition. They certainly consist in a decrease in rate of the fundamental activities of life, and if they go far enough either death or a break-up of the individual with reorganization and regressive changes occurs. For the present there seems to be ample justification for designating as physiological senescence



the progressive changes in condition associated with increase of size in Planaria and so for using size as a criterion of physiological age.

Table 1 shows the effect of this factor of size or physiological age upon head-frequency. Each pair of lots, I*a*, II*a*, etc., gives the percentages of the different types of head in corresponding pieces from animals of two different sizes. In every case the head-frequency is distinctly greater, usually much greater in the pieces from the larger (older) animals. The experiment has been performed many times with various sizes of animals and

pieces, being often used as a class experiment, and always with the same result, a higher head-frequency in the pieces from larger animals.

It may appear at first glance that these differences in head-frequency are due simply to the fact that in the longer older animals and the pieces of larger size there is more available

TABLE 1

Head-frequency in relation to physiological age (size)

	PIECE	NORMAL	TERATOPH-THALMIC	TERATO-MORPHIC	ANOPHTHAL-MIC	ACEPHALIC	DEAD
Series 339, 368. Pieces *A*, *B*, *C* (fig. 1), 50 each, from well fed animals: I, 7 mm. II, 20 mm.	I*A*	54	42			4	
	II*A*	86	14				
	I*B*		2			96	2
	II*B*	32	28	6	28	6	
	I*C*	4	12		2	76	6
	II*C*	34	14		26	26	
Series 321. Pieces *B*, *C* (fig. 1), 50 each, from well fed animals: I, 10–11 mm. II, 16–18 mm.	I*B*	6	12	6	26	50	
	II*B*	20	36	10	24	8	
	I*C*		6		8	86	
	II*C*	4	14	2	16	62	
Series 201, 277. Pieces *A*, *B*, *C*, *D* (fig. 2), 10 each, from well fed animals: I, 5–6 mm. II, 16 mm.	I*A*	60	40				
	II*A*	90	10				
	I*B*		20			80	
	II*B*	60	40				
	I*C*	10	40	10		30	10
	II*C*	90	10				
	I*D*	60	30				
	II*D*	100					

nutritive material for the development of a new head, but there are various reasons for believing that this is not the case: first, head-frequency is lowest in pieces from levels near the mouth where the amount of nutritive material in the pieces is greatest; second, it has been shown elsewhere that head-frequency may be altered experimentally in both directions without altering the amount of nutritive material (Child, '16; Behre,

'18), and further data bearing on this point are presented below; third, normal heads and often biaxial heads may develop on pieces so short that the whole substance is used up in the development of the head or heads. Taking all the facts into account, it is evident that while the amount of nutritive material may play some part in determining head-frequency, it is not the primary factor in determining the differences between large and small animals.

HEAD-FREQUENCY IN RELATION TO NUTRITIVE CONDITION

It has been shown that starvation in P. dorotocephala is accompanied by various changes in physiological condition. The susceptibility of body wall and ectoderm (probably also of parenchyma) increases from the beginning of starvation, that of the alimentary tract in the later stages (Child, '15 a, chap. VII, '19 c). Carbon dioxide production and oxygen consumption decrease in the early stages of starvation, undoubtedly because of the decrease in activity of the alimentary tract in the absence of food, but later increase (Child, '19 a; Hyman, '19 a, and in advanced stages of starvation the animal may have a much higher rate of respiration than at the beginning of starvation, and this rate is still further increased by renewed feeding. As regards rate of respiration and susceptibility, the starving animal apparently becomes physiologically younger, and with renewed feeding may again begin growth and progressive development from a stage physiologically earlier than that at the beginning of starvation. It is of interest, in the light of these facts, to determine the effect of starvation upon head-frequency, and data along this line are given in table 2.

Table 2 shows in all cases that the head-frequency in pieces from starved animals is less than in fed animals, even when of the same size.

Certain points in the various series demand brief notice. So far as actual difference in percentage is concerned, it will be observed that the effect of starvation is in general most conspicuous in the most anterior pieces (*A*) and in series 327 in the long pieces (*X*). The differences in these cases are entirely or almost en-

tirely between normal and teratophthalmic, and it has been found that very slight changes in physiological condition will determine the development of teratophthalmic instead of normal heads or vice versa. It is probable, therefore, that the large differences

TABLE 2

Head-frequency in relation to nutritive condition

	PIECE	NORMAL	TERATOPH-THALMIC	TERATO-MORPHIC	ANOPHTHAL-MIC	ACEPHALIC	DEAD
Series 368. Pieces *A*, *B*, *C* (fig. 1), 50 each	I*A*	10	90				
I, from animals 7–8 mm., starved 17 days	II*A*	54	42			4	
II, from animals 7–8 mm., well fed	I*B*				2	96	2
	II*B*		2			96	2
	I*C*		6			88	6
	II*C*	4	12		2	76	6
Series 327. Pieces *A*, *B*, *C*, *X* (fig. 1), 50 each	I*A*	20	72	2		2	4
I, from animals 6–7 mm., starved 19 days	II*A*	38	58			4	
II, from animals 6–7 mm., starved 18 days, fed once	III*A*	30	66				4
III, from animals 6–7 mm., heavily fed	I*B*		6	6	4	82	2
	II*B*			2	2	94	2
	III*B*	6	30	4	14	42	
	I*C*					92	8
	II*C*					100	
	III*C*			4		96	
	I*X*	30	70				
	II*X*	30	70				
	III*X*	70	18				12
Series 320. Pieces *B*, *C* (fig. 1), 50 each	I*B*			2	10	64	24
I, from animals 14–16 mm., starved 40 days	II*B*	4	46	6	30	14	
II, from animals 14–16 mm., well fed	I*C*			2	4	86	8
	II*C*	2	6	2	22	68	
Series 638. Pieces *A*, *B*, *C* (fig. 1), 50 each	I*A*	22	70	2	2	2	2
I, from animals starved 93 days, reduced from 25 mm. to 7–8 mm.	II*A*	88	8				4
II from animals 20 mm., well fed	I*B*		30	12	10	32	16
	II*B*	2	60	8	12	18	
	I*C*		4	4	10	82	
	II*C*		10	2	16	72	

in percentage in normal and teratophthalmic in the anterior pieces have no greater significance physiologically than smaller differences in percentages between headless and anophthalmic for example.

In series 368, 327, and 320 starved and fed animals and pieces are of equal size, but in series 638, well-fed large animals are compared with animals originally of still larger size, but reduced by starvation to a small fraction of the original size.

In all cases the comparative results are the same, the head-frequency being lower in the starved lot. In series 327 the animals of lot II are fed once after eighteen days of starvation and are compared with animals starved nineteen days (I) and heavily fed animals of the same size (III). Here the single feeding of lot II apparently increases head-frequency in *A*, but not elsewhere. The head-frequencies are low, even in the fed animals (III) of this series, except in the long pieces (*X*), because of the small size of the animals, but the difference between starved (I) and fed (III) is very great in *B* and *X*. That lack of available nutritive material is not the primary factor in determining the decrease in head-frequency in starvation is indicated by various facts: for example, the slight effect of the single feeding in series 327 II upon head-frequency indicates that some other factor than nutritive material is chiefly concerned. These pieces were well filled with food, at least during at least the earlier stages of regeneration. Moreover, all the series show that starvation and feeding do not alter essentially the relation between head-frequency and body-level, although there can be no doubt that in starvation nutritive reserves are exhausted first and resorption of the alimentary tract proceeds most rapidly in the anterior body regions. In other words, these regions are most starved, but still show a much higher head-frequency than the less starved regions near the mouth, and even after heavy feeding there is less surplus nutritive substance in the anterior body region, because of the alimentary tract is less extensively developed there than in regions about the mouth, yet anterior pieces show the highest head-frequency. In short, it is evident from these data on head-frequency in relation to starvation as well as from those on size

in the preceding section that amount of available nutritive material is not the primary factor in determining head-frequency. Further discussion is postponed to the final section of the paper.

HEAD-FREQUENCY IN RELATION TO MOTOR ACTIVITY OF PIECES

These experiments consist essentially in determination of head-frequency in pieces which are repeatedly stimulated to motor activity during a longer or shorter time beginning immediately after section (II) as compared with that of smaller pieces which are left undisturbed (I). The results for two series are given in table 3. In both cases the pieces *B* and *C* (fig. 1) are used because such pieces have neither an extremely high nor an extremely low head-frequency and change in both directions is readily possible. In *A* pieces and in pieces from the posterior zooids the head-frequency is usually so high that only decrease appears clearly in experiment. The methods of stimulation were various: currents of water were used to loosen the pieces from the glass, some motor activity usually following; individual pieces were loosened and turned over with the aid of a camel's-hair brush and usually attempted sooner or later to turn back again. Gentle stroking or slight pressure with a camel's-hair brush was also found to be effective. In series 302 such stimulation was repeated at least every hour for some eight hours after section, after which the pieces were left undisturbed overnight. On each day following until the new heads and eyes were distinctly visible the pieces were stimulated at least once an hour from 8.30 a.m. to 6 p.m. and at least twice between 8 and 11 p.m. In order to equalize oxygen supply as far as possible the water on the unstimulated lots (I) was stirred gently at the times when the other lots were stimulated, the stirring being usually not sufficient to induce motor activity.

In series 498 the stimulation of II was repeated every five to ten minutes during the first two hours after section, once an hour during the second two hours, and again every five to ten minutes during the third two hours. After this the pieces remained undisturbed. It was known at the time of this experiment that the continuation of the stimulation during several days as in

series 302 was wholly unnecessary, since under ordinary conditions it is determined within five or six hours after section whether a piece shall give rise to a head or not (Child, '14 d).

In table 3 the column 'teratophthalmic' is divided into two columns, *a* and *b*, which represent two different degrees of teratophthalmia. Column *a* includes the cases in which the two pigment spots of the eyes are distinct, but differ in size or are

TABLE 3

Head-frequency in relation to motor activity of pieces

	Piece	Normal	Teratophthalmic a	Teratophthalmic b	Terato-morphic	Anophthalmic	Acephalic	Dead
Series 302. Pieces *B*, *C* (fig. 1), from animals 16–18 mm., starved 8 days; I, undisturbed, water occasionally stirred; II, stimulated to motor activity as often as possible during 6 days after section	I*B*			8	8	26	58	
	II*B*		10	14	12	32	32	
	I*C*			4	4	12	80	
	II*C*	4	2	8	2	18	64	2
Series 498. Pieces *V*, *C* (fig. 1), from well-fed animals 16–18 mm.; I, undisturbed; II, stimulated to motor activity frequently during 6 hours after section	I*B*	4	38	44	6	8		
	II*B*	20	48	32				
	I*C*		22	34	4	20	22	
	II*C*	8	36	32		14	10	

somewhat asymmetrical in position. Column *b* includes those cases in which the two pigment spots are more or less approximated to each other and united by a band of pigment or partly fused. The forms of column *a* are somewhat nearer normal than those of column *b*.

The head-frequency data for these two series in table 3 show that in every case the stimulated lots (II) have a distinctly higher head-frequency, or more specifically, approach more closely to normality. The difference appears as clearly in columns *a* and

b under the head 'teratophthalmic' as in the other columns. In series 498 with well-fed animals the level of head-frequency is higher in all pieces than in series 302 with animals starved eight days and only moderately fed for several weeks previously, but the differences between I and II are distinct in every case. Moreover, the short period of stimulation in series 498 is apparently about as effective in increasing head-frequency as the long period in series 302. According to the evidence from head determination (Child, '14 d), this is to be expected, but it is of interest to find the data confirming expectation.

DISCUSSION

Extensive investigation of the conditions determining and affecting head formation in isolated pieces of Planaria has led to

Figure 3

certain conclusions concerning the physiological relations of the new head to other parts of the piece.

It is a well-known fact that regulatory development at the anterior end of a piece begins with the formation of a head, whatever the level from which the piece is taken, and that the parts which normally lie between the head and the level represented by the piece are formed later by reorganization of regions posterior to the new head and never develop unless at least a rudimentary head is formed first (Child, '11 c). In short, the head seems to arise as something more or less independent physiologically of other parts of the piece, while the further reogranization of the anterior regions of the piece occurs only under the influence of the new developing head.

Moreover, it has been shown that the relation between the cells from which the new head develops (fig. 3, *x*) and the rest of

the piece (fig. 3, y) is in a sense antagonistic, in that conditions which stimulate or accelerate the physiological activity of x in relation to y, or conversely, decrease the activity of y in relation to x increase head-frequency, while conditions which increase the activity of y in relation to x or decrease the activity of x in relation to y decrease head-frequency (Child, '14 d, '16). These relations have been expressed in the formula head-frequency $= \frac{\text{rate } x}{\text{rate } y}$. This formula is merely a brief expression of relations indicated by the experimental data. The physiological situation is apparently as follows: In an isolated piece certain cells (x) along the cut surface are isolated by the cut from all the correlative factors that formerly reached them from parts anterior to the cut and are also stimulated by the wound. These correlative factors represented a large part of the factors which determined the differentiation and behavior of these cells as a part of the body, and in their absence the cells tend to lose this differentiation and to become physiologically younger. But the relation of the cells x to the regions posterior to them (y) have not been altered by the cut, and any correlative factors which reach x from y must tend to prevent or retard its dedifferentiation and independent development. Consequently, the result in any particular piece will vary as one or the other of these factors has the ascendancy. For the present we may express this relation in terms of the activity of the regions x and y. If the activity (and probably the energy-liberating activity is primarily concerned) of x is sufficiently intense as compared with that of y, the cells of x will be in large measure independent of y and will dedifferentiate and develop anew in spite of y, and the product of this development will be a normal head; i.e., the primary developmental reaction of planarian protoplasm will occur (Child, '11 c, '15 b, pp. 96–102). If on the other hand, the activity of y in relation to that of x is sufficiently intense to inhibit or retard these processes to some extent, the development of the head will be retarded and its structure will range from a slight degree of teratophthalmia to extreme anophthalmia, or in the extreme case the development of a head is completely inhibited and the piece remains acephalic.

With this brief statement of the general conception of head-formation in pieces, we may turn to the interpretation of the data recorded above. As regards the relation between head-frequency and size, it was pointed out above that the rate of oxidation is unquestionably higher in the small than in the large animals. In isolated pieces of a smaller animal the tissue throughout is physiologically younger and more active, consequently the region x in its reaction to the absence of the parts in front and to the wound does not undergo so great an increase in rate in relation to y as in a piece of a larger older animal, and is therefore less independent of y in its development; i.e., shows a lower head-frequency than in the pieces from larger, older animals. From this point of view the lower head-frequency in the younger animals is essentially a consequence of their higher rate of metabolism or oxidation.

As regards the starved animals, it has been pointed out that lack of available nutritive material cannot be the primary factor in determining the lower head-frequency. The facts noted above concerning changes in susceptibility, CO_2 production and oxygen consumption during starvation indicate that the decrease in CO_2 production and oxygen consumption in the earlier stages are merely the result of the decrease in activity of the alimentary tract in the absence of food and that the rate of oxidation in ectoderm and body wall—probably also in the parenchyma—increases from the beginning of starvation. These are the regions chiefly concerned in the determination of head-frequency in pieces, and if the conclusions concerning rate of oxidation are correct, it is evident that the lower head-frequency in starving as compared with fed animals is due to difference in the relation $\frac{\text{rate } x}{\text{rate } y}$ of the same sort as in young animals as compared with old. In other words, the rate of energy-liberating metabolism being higher in the piece as a whole (fig. 3, y), the changes in the region x do not increase its rate over that of y so far as in well-fed or old animals. Consequently, in starving animals dedifferentiation and new development of x is less independent of correlative factors in y than in fed animals, and head-frequency is therefore lower.

In the pieces stimulated to motor activity, head-frequency is increased as compared with those at rest. The interpretation suggested for this fact is that the cells at the anterior end which are concerned in head-formation (fig. 3, x), are stimulated to a greater degree than the rest of the piece by the forward movement and that their independence of the correlative factors in y is therefore greater than in the pieces at rest. In other words, rate x undergoes increase in relation to rate y in this case and the result is increase in head-frequency.

It is clear, then, first, that the three physiological factors, age as indicated by size, nutrition, and motor activity, influence head-frequency; second, that this influence consists in increasing or decreasing the relative number of pieces in a lot which produce either heads or heads of a particular degree of development. As regards the various degrees of head-development, it is a fact of considerable interest that the different forms of head produced remain the same as regards their structure, whether such factors as size and region of piece (Child, '11 b, '14 b, '14 d), physiological age, nutrition, and motor activity or an external chemical agent such as KNC (Child, '16) or a physical agent such as temperature (Behre, '18) are concerned. It is impossible to escape the conclusion that the characteristics of the series of head forms from normal to acephalic are determined by the specific constitution of the protoplasm and that the effects of the various physiological conditions in altering head-frequency are essentially quantitative and non-specific. In the light of all these and various other facts, the quantitative interpretation presented above has gradually taken form and thus far no facts have been observed which are in conflict with it.

In conclusion, it may be pointed out that the tabulated data confirm earlier work on head-frequency in relation to length of piece and region of body (Child, '11 b, '16). In table 1 the pieces of series 201 and 277 are longer in relation to total length of body than the pieces of other series of the same table and the head-frequency is higher in these longer pieces. Similarly, the X pieces of series 327 in table 2, being longer than the A pieces of the same series, show a higher head-frequency than these, al-

though the anterior ends of both are at the same level of the body. The tables also show, as do the earlier data, that in pieces of equal length head-frequency decreases with increasing distance from the head of the animal, back to the level of the posterior zooid (Child, '11 d). Series 201 and 277 of table 1, the only series of this paper in which the posterior zooid is included, show an increase in head-frequency in the region of the posterior zooid. These relations between head-frequency and relative length of piece and region of body have been discussed and interpreted in the same terms as the relations between head-frequency and physiological condition considered in this paper (Child, '11 b, '14 b, '14 d) and recent work on carbon-dioxide production in pieces (Robbins and Child, '20) has added further evidence in support of the conclusions drawn from the earlier investigations.

SUMMARY

1. Head-frequency in the regeneration of pieces is lower in physiologically younger (smaller) than in physiologically older (larger) animals.

2. Head-frequency is lower in pieces from starved than in pieces from well-fed animals, even when the two are of the same size.

3. Head-frequency is higher in pieces which are frequently stimulated to motor activity during at least several hours after section than in pieces remaining undisturbed.

4. The range of head forms is the same in relation both to physiological conditions and to external chemical and physical agents, and the changes produced are changes in the frequency of the different forms. This non-specific effect of both physiological and external factors indicates that the action of these factors is essentially quantitative. It is shown that the quantitative interpretation of changes in head-frequency previously advanced serves for the facts presented in this paper.

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Resumen por el autor, E. C. McDowell.
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Herencia de las cerdas en Drosophila.

III. Correlación.

Los coeficientes de correlación y las lineas directas de regresión que indican el grado de semejanza entre los grados de cerdas de los padres y progenie en una raza de Drosophila, seleccionada durante cuarenta y nueve generaciones con el propósito de aumentar el número de cerdas, indican que en las primeras generaciones seleccionadas, los padres de grado elevado produjeron mas progenie de la misma clase que los de grado inferior; en las últimas generaciones no sucedió esto. Estos hallazgos están completamente de acuerdo con los efectos de la selección sobre los medios de la raza; en las primeras generaciones estos medios se elevaron por selección, mientras que en las generaciones ulteriores la selección no produce efecto alguno. Para probar finalmente el plasma germinativo presente en la raza seleccionada, se suspendió la selección a partir de la cuaranta y nueve generación, criándose un número elevado de moscas descendientes de un mismo par de la generación cuaranta y nueve bajo las mismas condiciones ambientes. Las correlaciones entre los padres y la progenie en las generationes cincuenta y dos y cincuenta y tres (en las que se hicieron mas de 31.000 numeraciones de cerdas) indican la completa ausencia de tendencia alguna en las moscas de grado elevado hacia la producción de descendientes de un grado mas elevado que el producido por los padres de grado mas bajo. El plasma generativo de la raza parece haberse transformado en uniforme por la selección e "inbreeding;" la primera ha reducido la cantidad de diferencias genéticas entre las células germinales; no hay prueba alguna de que diferencia genética alguna no presente al principio de los experimentos haya sido operativa.

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BRISTLE INHERITANCE IN DROSOPHILA[1]

III. CORRELATION

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EIGHT FIGURES

CONTENTS

INTRODUCTION

This paper is a coordinate part of the second paper in this series (MacDowell, '17 b) and would have followed it immediately had not the war intervened. The preceding report gave the details of the experiments and presented results in the form of means, standard deviations, and frequency distributions; this paper analyzes the same primary data by means of correlation tables. New data are presented, involving over 31,000 bristle counts, from four additional generations raised without selection as a final test of the germinal constitution of the selected race.

[1] Acknowledgment should be made of the cooperation of J. Gowen, J. Krafka and E. M. Vicari in the calculation of the constants in this paper, and especially of Miss Vicari's part in constructing the figures.

A summary of these results has already been presented (MacDowell, '17 a). Due to the lapse of time since the appearance of the second report, a brief résumé of the general results seems needed to introduce the new calculations.

PREVIOUS RESULTS

Extra dorsocentral bristles, in a certain race of Drosophila melanogaster, were found to act as a simple Mendelian character when crossed to normal wild flies. The number of these extra bristles varied, thus affording material for the employment of artificial selection. Starting from one pair of flies with extra bristles, and making brother by sister matings throughout, selections for increased bristle numbers were made for forty-nine generations. After the early generations, this selection did not modify the means of the race, atlhough the high limit of variation was far from being reached. Selections for decreased numbers of extra bristles at the beginning established a low race; similar selections from the later generations of the high-selected race were unsuccessful. Yet after a cross with normal these same generations of the high-selected race became immediately as amenable to low selection as were the unselected flies at the beginning. These general results were interpreted as being due to genetic differences among the germ cells of the original extra-bristled flies. These differences were quite independent of the single factor that controlled the appearance of any extra bristles. Selection reduced the number of these differences by reducing the amount of heterozygosis; crossing with normals increased these differences by increasing the amount of heterozygosis. The numbers of extra bristles that appear on a fly are influenced by external conditions as well as by genetic factors. The average number of extra bristles can be largely controlled by the amount of food a brood has a chance to eat before pupating; this amount depends upon the amount of food present and upon its attractiveness. This external influence naturally acts as a blind to the relationship between the grade and the genetic constitution of an individual, but it is obvious that this was not a complete blind, as there must have been

some connection between the degree of bristling and the genetic constitution before selection had started and again after a cross with normals.

It has been shown that only in the early generations are especially high parental averages associated with exceptionally high filial averages. Since the offspring can never be raised under an environment identical with that of the parents, the failure to find any correspondence between the means of the parents and offspring in later generations may be due to the different environments in successive generations. However, the highest-grade flies may have been produced by the highest-grade parents, however well the environment may have concealed such a relationship between the means when different generations were compared. The whole frequency distribution of the offspring may be centered about quite a different mean from that of the parents, yet the relative positions of parents and offspring in their own distributions may be the same. The study of the relationship between parents and offspring in individual families offers a different line of attacking the problem. Such an approach goes directly to the heart of the question that selection seeks to answer empirically: are there genetic differences between flies with different numbers of extra bristles and, if so, do such differences arise continuously? If the grades of the parents and offspring bear any direct relationship to each other, selection can progress; if such a relationship appears at first and then disappears, one may conclude that selection will not continue to be successful. The demonstration that these are the facts of the relationships between parents and offspring in the various selected generations will strengthen considerably the conclusions drawn from the results of selection based on the means.

CORRELATION

The obvious method of investigating the relationship between the parents and offspring, is that of the correlation table, with the coefficients of correlation and regression calculated from it. This method affords a clear description of large masses

of data that would otherwise be difficult, if not impossible, to summarize. The relationship of every fly to its parents has its effect on the results. However, correlation tables afford only superficial descriptions, and accordingly great care is necessary in determining their meaning. A positive correlation coefficient indicates that higher parents had higher-grade offspring; a negative coefficient indicates that higher-grade parents had lower-grade offspring. But from any single coefficient little can be said of the underlying genetic significance. In any particular generation the high-grade parents may have had, on the whole, better conditions than the low-grade parents and consequently produce higher-grade offspring; this would give a positive coefficient. The reverse might be equally possible in another generation; the lower-grade parents might, by chance, have found better conditions and so have produced higher-grade offspring than the high parents; in such a case the correlation would be negative. Although a single plus or minus coefficient would not bear much evidence, a series of one or the other would indicate that real genetic phenomena were involved. Even such a series cannot be authoritative unless the experimental procedure has been the same for all families included. This is one of the greatest difficulties with results based alone on mathematical treatment. In many such cases the material has lacked homogeneity. In this respect the bristle data have one great advantage, being derived entirely from experimental procedure; their origin and the nature of the families put together are fully known.

The selection of low-grade flies tends to isolate the small ones and therefore to carry on any conditions that tend to make small flies, such as weakness or disease. Such weakness, apart from any germinal cause, would tend to make the offspring of these low-selected parents lower than the offspring of parents selected for grades not associated with weakness. If such differently selected lines be united in one correlation table, even though they are all the result of brother and sister matings and are all the same number of generations away from common ancestors, the finding of a positive coefficient would

not prove that genetic differences exist between high and low flies. Thus there are two non-genetic factors that may occasion positive correlation coefficients: environment and lack of vigor due to the continued selection of small flies.

Methods

In the correlation tables nothing but the progeny of one original pair of flies has been included. As indicated, the return-selected lines probably tend to increase the amount of correlation unduly. However, one of these lines has been included in the tables, namely, the line started from the 16th generation of the high race; two generations of the second return line, started from the 27th generation, have also been included. The later generations of this last line consisted of such obviously inferior flies that they were omitted. The line of low-grade selection started at the beginning has also been included. The essential genetic difference between this low race and the two return-selected races has been pointed out. There is no suggestion that the low-selected race at the beginning had any lack of vigor; the means were immediately influenced and there was no question as to the distinctness of the means from those of the high race. On the other hand, the first few generations of the return-selected races showed no influence at all of the selection, but there did gradually appear a slight relative lowering of the means, which may well be due to accumulated weakness. The inclusion of these two sorts of low-selected lines will make the comparison of the correlation in the corresponding periods more fair.

In the tables the families were arranged according to the mean bristle grades of the parents. Since in most cases the parents were of the same, or within one bristle of the same grade, the averages were only slightly different from the actual grades. Sons and daughters were tabulated separately. The tables themselves are too numerous for publication in a journal. The correlation coefficients, their probable errors and the ratios of the errors to the coefficients are given in table 1.

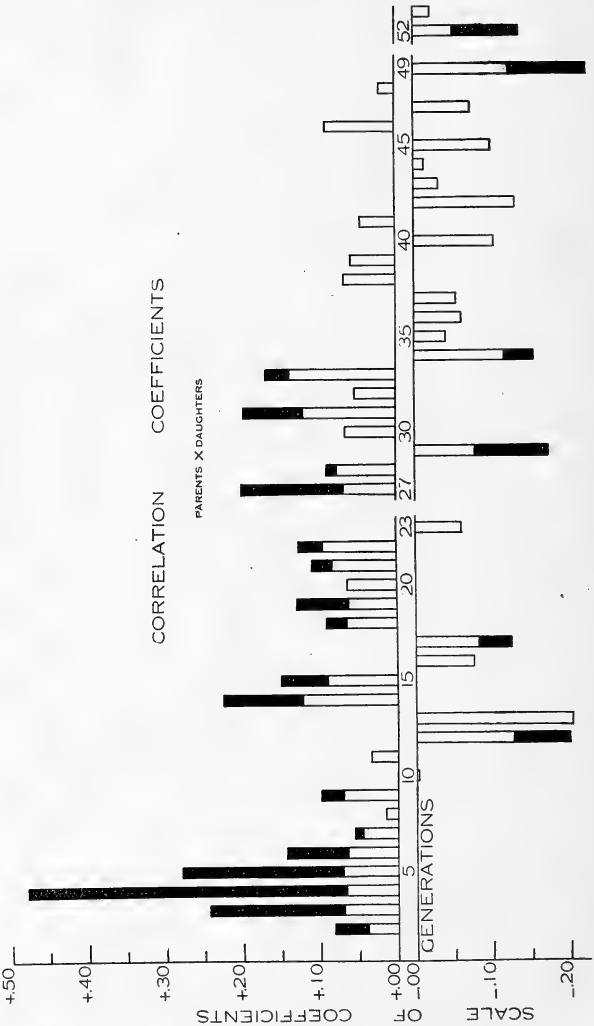
TABLE 1

Means and numbers of parents, means, numbers, correlation coefficients, parents by offspring, probable errors and ratios of errors to coefficients of the sons and daughters in all the generations of the inbred race; probable errors based on the mean squares of the numbers of parents and children

GENERATION	PARENTS			SONS				DAUGHTERS			
	Numbers	Means[1]	Range	Numbers	Means	Correlation coefficients	CRR/E_r	Numbers	Means	Correlation coefficients	CRR/E_r
2	72	1.9586	4	3586	1.5652	+0.0567±0.0133	4.26	3928	1.9480	+0.0819±0.0127	6.44
3	30	3.4663	6	1006	1.0965	+0.2026±0.0242	8.37	1089	2.7098	+0.2433±0.0229	10.62
4	26	4.0968	6	771	2.2243	+0.3723±0.0249	14.95	777	2.9292	+0.4789±0.0221	21.66
5	26	4.2602	7	891	2.1885	+0.1591±0.0262	6.07	982	3.1598	+0.2798±0.0236	11.85
6	32	4.4932	8	1327	2.7008	+0.0592±0.0219	2.07	1356	4.0103	+0.1438±0.0213	6.75
7	74	5.1485		3010	2.4730	+0.0338±0.0146	2.31	2968	3.7823	+0.0565±0.0147	3.84
8	34	5.6248	12	1132	2.7376	+0.1026±0.0236	4.34	1219	4.0877	+0.0154±0.0229	0.67
9	28	5.4379	4	871	2.8530	−0.0100±0.0271	0.37	921	4.0130	+0.0985±0.0261	3.77
10	50	5.6786	6	1238	2.6316	−0.0349±0.0227	1.53	1191	3.7791	−0.0030±0.0232	0.12
11	40	5.9336	6	1453	2.5113	+0.0426±0.0210	2.03	1451	3.5037	+0.0347±0.0210	1.65
12	10	5.8252	5	268	2.7835	−0.2698±0.0453	5.95	330	4.1545	−0.1992±0.0423	4.70
13	16	5.2802	5	81	3.2469	−0.0120±0.0885	0.13	117	4.8376	−0.2044±0.0709	2.88
14	6	5.6983	6	293	2.8054	+0.01261±0.0461	2.73	360	4.1222	+0.2247±0.0402	5.58
15	18	6.0632	6	741	2.3724	+0.0355±0.0294	1.20	737	3.7842	+0.1507±0.0294	5.12
16	26	4.8717	9	621	2.2640	−0.0395±0.0321	1.23	669	3.5515	−0.0766±0.0308	2.48
17	24	4.5368	7	894	2.4149	+0.0083±0.0268	0.30	842	3.5629	−0.1258±0.0272	4.62
18	32	4.4419	9	1286	2.3048	+0.1027±0.0221	4.64	1349	3.3232	+0.0911±0.0213	4.27
19	42	5.0053	7	1531	2.4663	+0.0880±0.0203	4.33	1488	3.4213	+0.1293±0.0204	6.33
20	20	5.2573	8	528	2.5814	+0.0739±0.0347	2.13	560	3.6928	+0.0645±0.0337	1.91
21	20	5.1408	7	748	3.0160	+0.1348±0.0288	4.68	825	4.3466	+0.1090±0.0276	3.94
22	22	4.5052	8	583	3.0686	+0.0358±0.0331	1.08	629	4.4069	+0.1271±0.0314	4.04
23	10	6.1648	7	251	3.3107	−0.0324±0.0505	0.64	207	4.7777	−0.0609±0.0554	1.09

24–25–26[2]											
27	22	3.1437	5	1053	2.9021	+0.1415±0.0242	5.84	1125	4.3253	+0.2013±0.0229	8.79
28	24	5.5486	9	775	3.0774	+0.1192±0.0284	4.19	972	4.4485	+0.0908±0.0255	3.56
29	24	6.7969	11	807	3.9876	−0.1012±0.0279	3.62	931	5.7776	−0.1751±0.0258	6.78
30	20	6.7627	9	395	4.3417	+0.0391±0.0402	0.97	488	6.3094	+0.0669±0.0361	1.85
31	14	8.2845	9	328	4.0365	+0.1549±0.0432	3.58	373	5.7211	+0.1988±0.0398	4.99
32	48	5.9558		808	3.2500	+0.1305±0.0277	4.71	710	4.6169	+0.0537±0.0300	1.79
33	14	6.8106	4	196	3.5408	+0.0598±0.0551	1.08	208	4.6923	+0.1692±0.0540	3.13
34	18	6.1615	4	442	3.0610	−0.2010±0.0366	5.49	412	4.5121	−0.1557±0.0385	4.04
35	14	6.6972	5	296	3.2770	−0.0076±0.0466	0.16	297	4.2020	−0.0412±0.0464	0.88
36	18	6.4652	6	343	3.2769	−0.1554±0.0422	3.68	347	4.7233	−0.0611±0.0428	1.42
37	36	6.4727	5	555	3.7099	+0.0088±0.0340	0.25	637	5.0329	−0.0534±0.0316	1.68
38	28	6.2369	4	489	3.8159	+0.0148±0.0362	0.48	490	5.2693	+0.0676±0.0360	1.87
39	30	6.5862	5	427	3.6557	−0.1274±0.0381	3.34	483	5.1739	+0.0588±0.0363	1.61
40	24	6.6167	3	151	3.5165	+0.1303±0.0638	2.04	200	4.8950	−0.1030±0.0560	1.83
41	18	6.4857	5	296	4.0608	+0.0710±0.0464	1.53	264	5.5909	+0.0459±0.0492	0.93
42	14	6.8542	5	231	3.1471	+0.0033±0.0526	0.06	290	4.3862	−0.1314±0.0463	2.83
43	42	6.0825	5	730	3.6397	+0.0366±0.0296	1.23	765	5.1477	−0.0324±0.0289	1.12
44	22	6.7363	6	389	3.5424	−0.1062±0.0401	2.64	383	4.8903	−0.0137±0.0409	0.33
45	22	6.4876	4	172	3.9825	−0.0552±0.0706	0.78	192	6.1562	−0.0992±0.0570	1.74
46	18	6.6271	5	262	3.3893	−0.0002±0.0494	0.00	328	4.9451	+0.0911±0.0439	2.07
47	20	5.6631	4	222	3.7072	−0.0956±0.0531	1.80	250	5.0640	−0.0743±0.0504	1.47
48	30	5.9537	6	398	3.8442	−0.1208±0.0396	3.05	445	5.0292	+0.0210±0.0380	0.55
49	18	7.3656	6	248	3.0564	−0.1019±0.0503	2.02	344	4.3255	−0.2242±0.0410	5.46
50–51[2]	(all selection suspended after generation 49)										
52	44			2169	2.7270	−0.1436±0.0266	5.30	2195	3.9052	−0.1378±0.0168	8.20
53	296			13210	2.6598	−0.0271±0.0069	3.92	13923	3.7842	−0.0221±0.0068	3.25

[1] Weighted according to numbers of offspring. [2] Too few families for the calculation of coefficients.


CORRELATION COEFFICIENTS
PARENTS X DAUGHTERS
GENERATIONS
SCALE OF COEFFICIENTS
+.50
+.40
+.30
+.20
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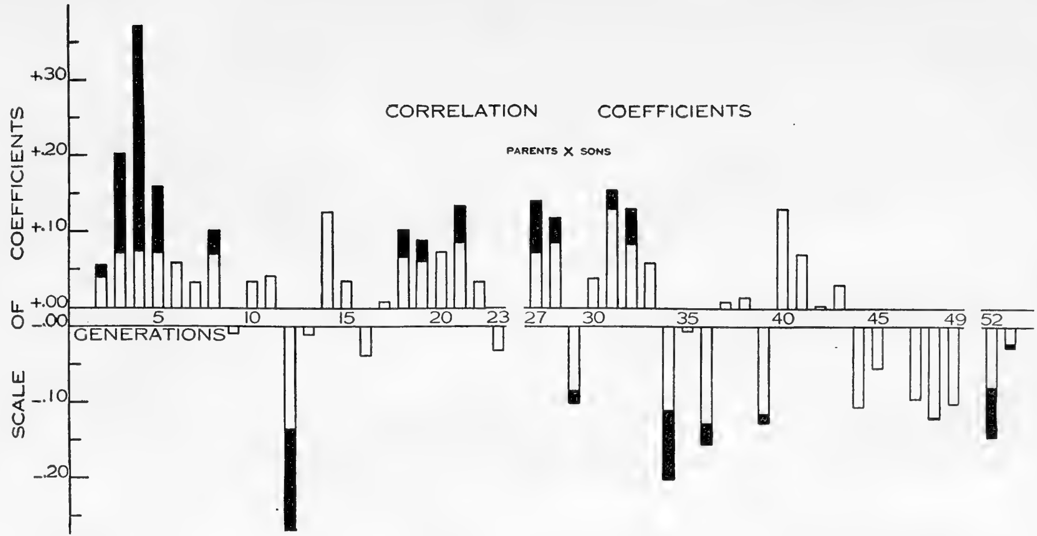
1A



1B

Fig. 1 *A*, Correlation coefficients for daughters by parents; *B*, sons by parents. Bars above the base line indicate plus coefficients, those below, minus coefficients. Solid black portions of the bars indicate the amount the coefficient exceeds three times its probable error; bars with no black portion are not statistically significant.

In figures 1, A and B, the correlation coefficients for the sons and daughters are presented graphicly. Bars above the baseline indicate plus coefficients, those below, minus coefficients. The breaks in the base line call attention to the generation omitted because they included too few families for the calculations of the coefficients. The solid black portions of the bars indicate the amount the coefficient exceeds three times its probable error; accordingly, bars with no black portion represent coefficients that are not statistically significant. Before discussing the actual findings, the manner of calculating the probable errors should be explained. Clearly probable errors have the greatest importance in evaluating any statistical result. In calculating correlation coefficients involving few parents and many offspring, the numbers of parents are weighted according to the numbers of their offspring. However, there are fewer parents, and the probable errors based on the numbers of offspring are therefore smaller than the facts would justify. If, on the other hand, the actual number of parents is used, the errors will be far too large. Sturtevant ('18, p. 10) has stated the case clearly; he gives the errors based upon the numbers of offspring, but does not consider that his correlation coefficients have much significance. In the present case the errors have been based on the mean squares of the numbers of parents and offspring:

$$\sqrt{\frac{n_1^2 + n_2^2}{2}}$$

in which n_1 is the number of parents and n_2, the number of offspring. This of course does not solve the difficulty, but the errors seem to be more reasonable than those obtained by other methods. The errors by both other methods have been calculated; the ones given are not enough greater than those based on the numbers of offspring to change the general appearance of the charts; the errors based on the numbers of parents considerably reduce the number of significant coefficients, but the same conclusions are to be drawn whatever set of errors is used. Figure 2, showing the empirical means and the regression straight lines, should be studied in connection with these

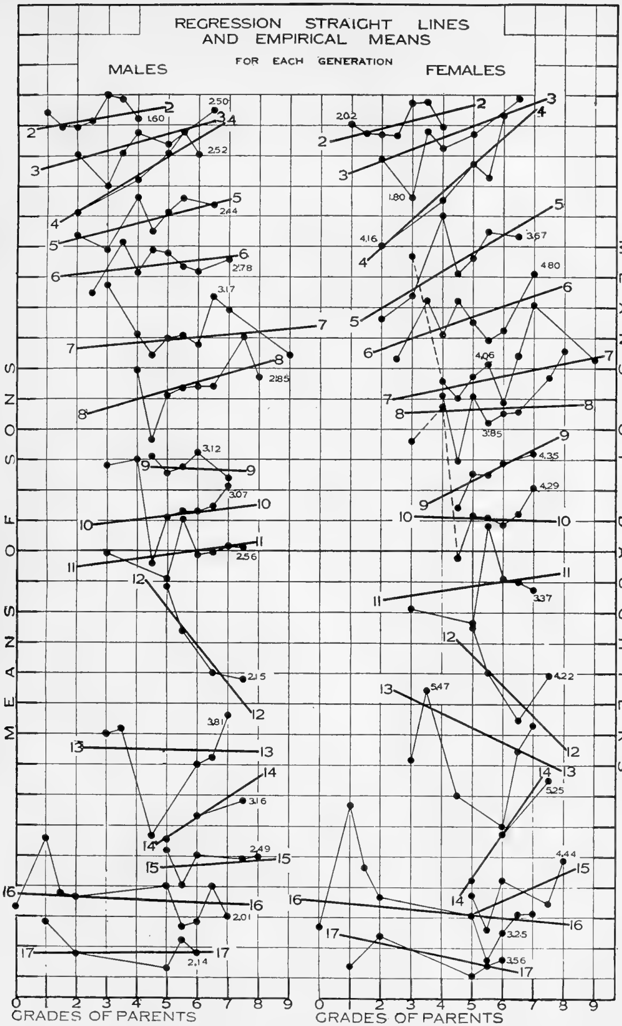


Figure 2, A

Fig. 2, *A*, *B*, *C* Regression straight lines and empirical means calculated from the data in table 1, formulae in table 2. Each dot represents the mean of the sons or daughter that were produced by parents of the grade indicated on the scale at the bottom of the figure. One bristle is the unit of the scale of means. Since the scales of means for different generations overlap, the absolute values of the empirical means cannot be given on the scales.

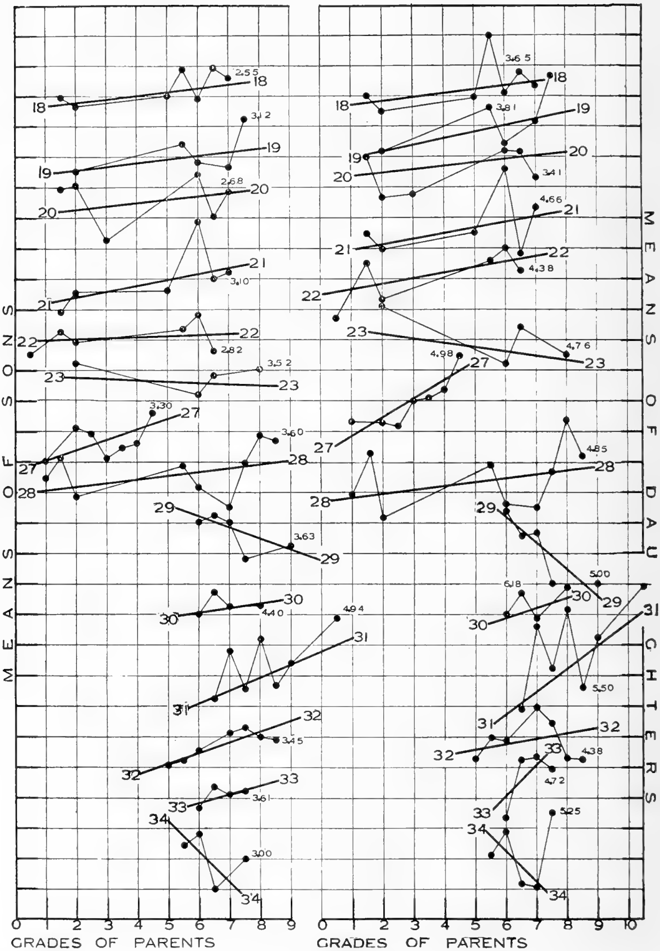

MEANS OF SONS
MEANS OF DAUGHTERS
GRADES OF PARENTS
GRADES OF PARENTS

Figure 2, B

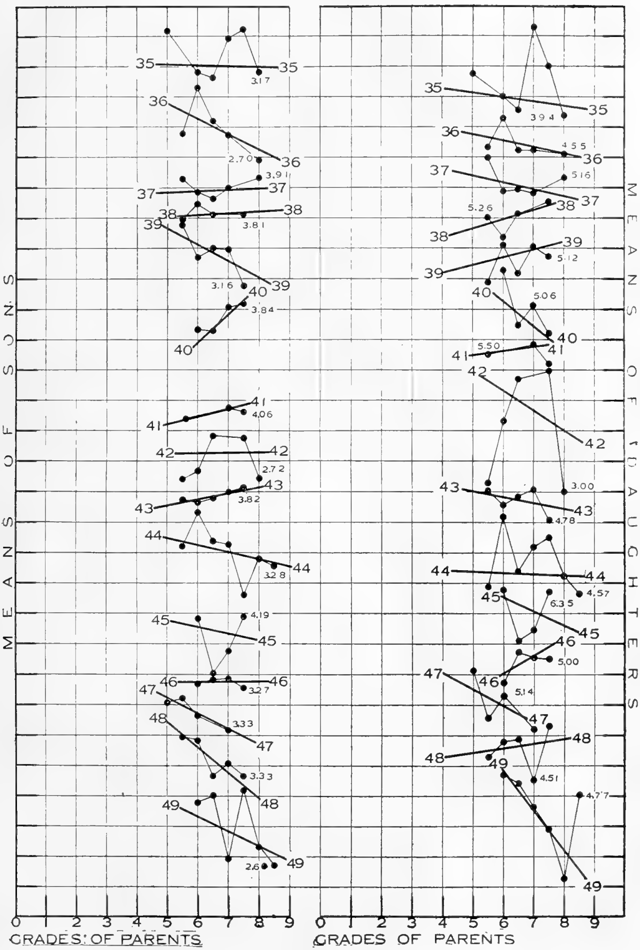

MEANS OF SONS
MEANS OF DAUGHTERS
0 1 2 3 4 5 6 7 8 9
GRADES OF PARENTS
0 1 2 3 4 5 6 7 8 9
GRADES OF PARENTS

Figure 2, C

correlation coefficients. Each dot represents the empirical mean of the sons or daughters that came from parents of the grade indicated on the scale at the bottom of the chart. To conserve space, the scales of the means for the different generations have been overlapped, so no absolute values can be indicated on these scales. To indicate absolute values, the numbers are given for one empirical mean about each straight line; these numbers are in terms of extra bristles; they are given with two places in the decimal and are in smaller type than the numbers indicating the generations. In this way the absolute values of the other empirical means can be estimated.

The slant of the line is a function of the correlation coefficient; if the line rises at the right, positive correlation is indicated; if it is horizontal, there is no correlation; if the line falls at the right, negative correlation is indicated. The relations of the empirical means to the regression straight lines form a check on the statistical result. The correlation coefficients would be accurate descriptions if the empirical means fell upon these lines; in this case the line connecting the successive means for one generation would be straight and would coincide with the regression lines. This arrangement of the empirical means shows clearly the direct relationship between the parents and offspring with no complicating mathematical treatment involved.

Results

If the generations that show the most unquestioned correlation be considered alone, the following statements may be made: in thirteen out of forty-seven cases the correlation is large enough to be significant for both males and females in the same generation. Of these thirteen correlations, ten are positive and three negative; the only four of these ten positive correlations that appear in consecutive generations come in generations 2 to 5; the only other consecutive ones are in generations 18 and 19 and 27 and 28. The average of the ratios of errors to coefficients in the four generations 2 to 5 is practically twice as large as the corresponding average in the other six positive generations:

TABLE 2

Straight line equations showing the regression of grade of offspring upon grade of parent per generation. S—grade of son; D—grade of daughter; p, grade of parents

GENERATIONS	SONS	DAUGHTERS	GENERATIONS	SONS	DAUGHTERS
2	$S = 1.4003+0.0422\,p$	$D = 1.6988+0.0634\,p$	27	$S = 2.3571+0.0868\,p$	$D = 3.3585+0.1534\,p$
3	$S = 1.4357+0.0683\,p$	$D = 2.0391+0.0961\,p$	28	$S = 2.7329+0.0309\,p$	$D = 4.0971+0.0317\,p$
4	$S = 0.9744+0.1516\,p$	$D = 1.0718+0.2279\,p$	29	$S = 5.2235-0.0915\,p$	$D = 8.6978-0.2167\,p$
5	$S = 1.6228+0.0658\,p$	$D = 1.8815+0.1511\,p$	30	$S = 3.8123+0.0392\,p$	$D = 5.1761+0.0836\,p$
6	$S = 2.4433+0.0286\,p$	$D = 3.2348+0.0861\,p$	31	$S = 2.2512+0.0372\,p$	$D = 2.6281+0.1856\,p$
7	$S = 2.2267+0.0239\,p$	$D = 3.2398+0.0527\,p$	32	$S = 2.2124+0.0866\,p$	$D = 4.0912+0.0443\,p$
8	$S = 1.9342+0.0714\,p$	$D = 3.9521+0.0120\,p$	33	$S = 2.5627+0.0717\,p$	$D = 1.1155+0.2626\,p$
9	$S = 2.9656-0.0102\,p$	$D = 2.6297+0.1279\,p$	34	$S = 6.1342-0.2507\,p$	$D = 7.5771-0.2472\,p$
10	$S = 2.2991+0.0293\,p$	$D = 3.8290-0.0043\,p$	35	$S = 3.3439-0.0049\,p$	$D = 4.7044-0.0375\,p$
11	$S = 2.1044+0.0344\,p$	$D = 3.0609+0.0371\,p$	36	$S = 4.9230-0.1269\,p$	$D = 5.4431-0.0558\,p$
12	$S = 6.4110-0.3096\,p$	$D = 7.1090-0.2547\,p$	37	$S = 3.6056+0.0079\,p$	$D = 5.7928-0.0293\,p$
13	$S = 3.3085-0.0055\,p$	$D = 6.0449-0.1184\,p$	38	$S = 3.6264+0.0152\,p$	$D = 4.3261+0.0754\,p$
14	$S = 0.8855+0.1673\,p$	$D = 0.0658+0.3695\,p$	39	$S = 5.4245-0.1338\,p$	$D = 4.3375+0.0636\,p$
15	$S = 2.1351+0.0194\,p$	$D = 2.4311+0.0194\,p$	40	$S = 0.4387+0.2312\,p$	$D = 7.5943-0.2048\,p$
16	$S = 2.3712-0.0106\,p$	$D = 3.7635-0.0224\,p$	41	$S = 3.3271+0.0563\,p$	$D = 5.1075+0.0374\,p$
17	$S = 2.3862+0.0031\,p$	$D = 4.0445-0.0535\,p$	42	$S = 3.1069+0.0029\,p$	$D = 6.5952-0.1617\,p$
18	$S = 2.0383+0.0304\,p$	$D = 3.0360+0.0318\,p$	43	$S = 3.0649+0.0472\,p$	$D = 5.7269-0.0476\,p$
19	$S = 2.1742+0.0292\,p$	$D = 2.8830+0.0536\,p$	44	$S = 4.3312-0.0584\,p$	$D = 5.0154-0.0093\,p$
20	$S = 2.2930+0.0273\,p$	$D = 3.3938+0.0285\,p$	45	$S = 4.7201-0.0567\,p$	$D = 7.6633-0.1163\,p$
21	$S = 2.5263+0.0471\,p$	$D = 3.8747+0.0463\,p$	46	$S = 3.3926-0.0002\,p$	$D = 3.0462+0.1438\,p$
22	$S = 2.9817+0.0097\,p$	$D = 3.9907+0.0449\,p$	47	$S = 5.1540-0.1281\,p$	$D = 6.5957-0.1348\,p$
23	$S = 3.4377-0.0106\,p$	$D = 5.2290-0.0351\,p$	48	$S = 6.3302-0.2105\,p$	$D = 4.6535+0.0388\,p$
24–25–26			49	$S = 4.9370-0.1255\,p$	$D = 9.0537-0.3249\,p$

Averages of r/E_r

GENERATIONS	MALES	FEMALES
2, 3, 4, 5	8.41	12.64
18, 19, 21, 27, 28, 31	4.54	5.31

From direct observation of figure 1 it appears that three groups of generations may be roughly distinguished: group 1, generations 2 to 10; group 2, generations 11 to 34; group 3, generations 35 to 49. In the first group the correlation coefficients are generally significant and positive; in the second group the coefficients fluctuate, their values are smaller and, in some generations, significantly negative; in the third group, the only significant coefficients are negative.

Since the second group of generations involves the only uncertain results, it should be especially examined. Positive correlation is surely present, but so is significant negative correlation. From figure 2 it will be observed that especially low-grade parents were among those used in generations 16 to 23. These formed the return-selected line already mentioned. The means of this line are generally parallel with the means of the high-selected race during the corresponding period (MacDowell, '17, p. 125 and fig. 7), yet there is a tendency for the means of the return-selected line to fall a little lower. As already indicated, this difference in the means is probably due to the unconscious selection of a weak race.

A few unpublished data on the relation of the size of the fly to the bristle numbers may well be presented at this point. Sixty-four small flies from an old, dried-up bottle were turned into a new bottle whose conditions proved to be especially favorable. When the bottle was next examined the new generation had begun to hatch. The offspring were so much larger and brighter colored than the parents that there was not the slightest doubt as to which generation each fly belonged. Fifty-one flies had hatched in the next generation; their average weight was 0.000943 gram; the average weight of the parents at this time was 0.000310. The bristle counts of the two groups were as follows:

Extra bristles

	0	1	2	3	4	5	6
Parents	28	25	11				
Offspring		4	9	12	16	9	1

If the means of the high and the return-selected race differ on account of non-genetic causes, the positive correlation coefficients obtained when the two lines are correlated at the same time are due to these same non-genetic causes. For it can be shown that the high-selected line within itself at this time did not produce higher-grade offspring from higher-grade parents. In figure 3 and table 3 are given the correlation coefficients for a period covering the time of the return-selected race.

In order to group together flies with more similar environments, the families are arranged according to the time of mating (in half-month periods) instead of by generations. Groups 1 to 10 correspond in time to the return-selected line. The sons do not show a single group with significant positive correlation; the daughters show just one such group. So it is evident that the significant positive correlation shown in generations 18 to 23 in figure 1 does not mean that the parents in these generations of the high-selected race differ genetically. This conclusion holds whether the non-genetic interpretation of the lowered means in the return-selected race is accepted or not.

Generations 24, 25, and 26 include but one family each. The breeding conditions were very unfavorable at this time. To save the race all the flies available in generation 26 were mated, however low their bristle grades. Soon after this the bottles were transferred to a constant-temperature room (90°F.); the size and bristle number of the flies at once rose higher than at any other time (MacDowell, '17, fig. 3, p. 114). Unfortunately, all the matings for one generation were not then made at the same time, so different generations entered the warm room at the same time. The families in the second half of this middle period, when grouped by generations show four positive and two negative correlation coefficients. When arranged by half-month periods, and the return selections omitted, as shown

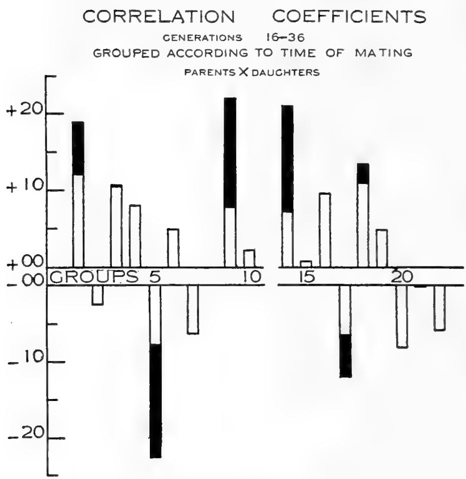


Figure 3, A

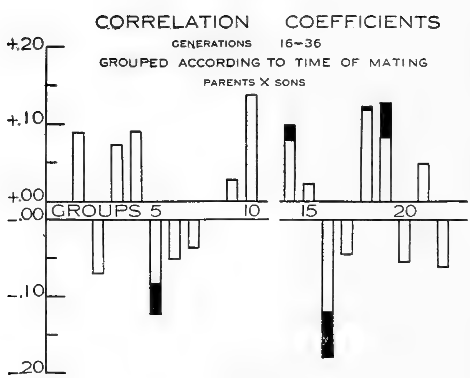


Figure 3, B

Fig. 3, A and *B* Correlation coefficients, sons and daughters by parents in the high-selected race alone; families grouped by the time of mating. *A*, daughter by parents; *B*, sons by parents. Plotted as in figure 1.

TABLE 3

Correlation coefficients, sons and daughters by parents; families grouped according to the time of mating, in half-month periods. Data approximately correspond to generations 16 to 35 of the high-selected line

HALF MONTH	PARENTS	SONS				DAUGHTERS			
	Numbers	Numbers	Means	Correlation coefficients	r/E_r	Numbers	Means	Correlation coefficients	r/E_r
1	14	366	2.1912	+0.0898±0.0416	2.15	373	3.4075	+0.1896±0.0400	4.74
2	16	617	2.5040	−0.0709±0.0321	2.20	558	3.5860	−0.0254±0.0342	0.74
3	20	512	2.2402	+0.0738±0.0352	2.09	522	3.4310	+0.1063±0.0347	3.06
4	8	489	2.4867	+0.0913±0.0359	2.54	536	3.3526	+0.0800±0.0344	2.32
5	20	798	2.5175	−0.1236±0.0279	4.43	842	3.4453	−0.2274±0.0262	8.67
6	12	461	2.7288	−0.0527±0.0372	1.41	420	3.8571	+0.0496±0.0390	1.27
7 and 8	10	426	2.7065	−0.0363±0.0388	0.93	426	4.1197	−0.0647±0.0387	1.67
9	16	775	3.0864	+0.0289±0.0288	1.00	877	4.3797	+0.2212±0.0257	8.60
10	12	237	3.2911	+0.1399±0.0510	2.74	220	4.8454	+0.0234±0.0539	0.43
11, 12, 13	Too few families								
14	16	920	2.9619	+0.0999±0.0263	3.79	988	4.3815	+0.2126±0.0243	8.74
15	12	498	2.9437	+0.0236±0.0359	0.65	576	4.2343	+0.0076±0.0334	0.22
16	10	270	3.5000	−0.1861±0.0483	3.85	350	4.9771	+0.0967±0.0424	2.28
17	38	1128	4.0425	−0.0452±0.0238	1.89	1328	5.8855	−0.1215±0.0216	5.62
18	20	401	4.1022	+0.1247±0.0394	3.16	464	5.9094	+0.1347±0.0365	3.69
19	46	808	3.2549	+0.1283±0.0277	4.63	706	4.6147	+0.0487±0.0300	1.62
20	28	574	3.2456	−0.0552±0.0333	1.65	578	4.6211	−0.0828±0.0331	2.50
21	20	355	3.2140	+0.0501±0.0424	1.18	348	4.1867	−0.0023±0.0429	0.53
22	54	913	3.5487	−0.0627±0.0264	2.37	1004	4.9292	−0.0599±0.0258	2.32

in figure 3 and table 3, groups 14 to 20 show considerably reduced correlation. It seems probable that if the environment had been ruled out still further by making all the matings on the same day, as was done for all the generations beginning with the 36th, there would be found no more correlation in this period than in the period beginning with generation 36.

Although lumping data often conceals much that is important, it also tends to balance out fluctuating differences. After making the above study of individual generations in a somewhat detailed manner, the results of correlating the generations in groups of five may be presented. All the same data

TABLE 4

Correlation coefficients, parents by sons and daughters; generations put together in groups of five. The same primary data as used in table 1

GENERATIONS	MALES			FEMALES		
	Numbers	Coefficients	r/E_r	Numbers	Coefficients	r/E_r
2– 6	7581	+0.3443±0.0081	42.50	8132	+0.4855±0.0068	71.39
7–11	7704	+0.0400±0.0090	4.44	7750	+0.0228±0.0091	2.50
12–16	2004	−0.0029±0.0100	0.29	2213	+0.0147±0.0170	0.88
17–21	4987	+0.1063±0.0110	9.66	5064	+0.0880±0.0111	7.92
22–26	906	+0.0361±0.0266	1.35	921	+0.1131±0.0261	4.33
27–31	3358	+0.3002±0.0126	23.82	3889	+0.2952±0.0117	25.23
32–36	2085	+0.0329±0.0175	1.88	1974	−0.0146±0.0180	0.81
37–41	1918	−0.0192±0.0183	1.04	2074	+0.0033±0.0176	0.18
42–46	1784	−0.0584±0.0189	3.08	1958	−0.0757±0.0162	4.67
47–49	868	−0.2350±0.0257	9.14	1039	−0.2016±0.0238	8.47

used for the correlations for single generations were used. The constants are given in table 4.

This grouping gives much larger numbers and accordingly smaller probable errors; it greatly increases the variations in the environment involved in each coefficient, so there is more chance of their being balanced out than when shorter periods were included. The regression straight lines and empirical means in figure 4 and table 5 indicate that the correlation coefficients for these groups give a somewhat more accurate description of the general relationship of parents and children; the means lie more closely along the regression lines as would

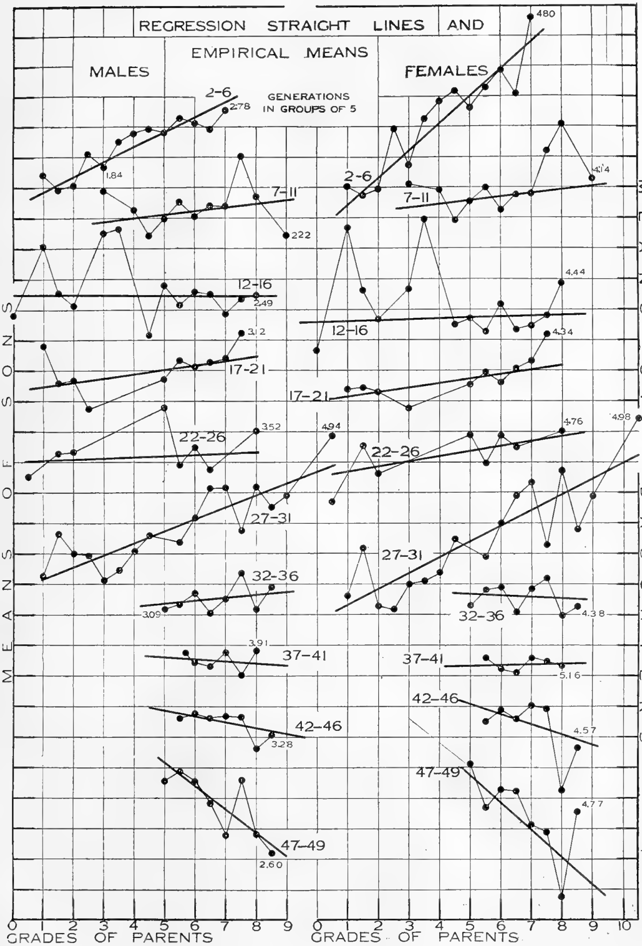


Fig. 4, See legend for fig. 2. Formulae for regression lines in table 5

be expected from larger numbers. The three periods marked out above stand out clearly (fig. 5). The highest degree of correlation appears in the first period; the middle period gives strong correlation in generations 27–31, with smaller amounts in the two preceding groups; the third period shows no correlation at all. With no knowledge of the influences back of the coefficients in the middle period, it would be easy to assume that these positive coefficients indicate genetic variability, whereas according to the preceding discussion it appears that they are probably due to non-genetic causes.

TABLE 5

Straight-line equations showing the regression of grade of offspring upon grade of parents; generations in groups of five. S, grade of son; D, grade of daughter; p, grade of parents

GENERATIONS	REGRESSION EQUATIONS	
	Sons	Daughters
2– 6	S = 1.1759+0.1252 p	D = 1.2506+0.2255 p
7–11	S = 2.2720+0.0288 p	D = 3.4697+0.0305 p
12–16	S = 2.5065−0.0012 p	D = 3.8001+0.0072 p
17–21	S = 2.1665+0.0356 p	D = 3.2531+0.0358 p
22–26	S = 3.0147+0.0101 p	D = 4.0144+0.0421 p
27–31	S = 2.4054+0.0982 p	D = 3.6718+0.1272 p
32–36	S = 2.9444+0.0239 p	D = 4.7264−0.0133 p
37–41	S = 5.6872−0.1480 p	D = 5.1323+0.0036 p
42–46	S = 4.1747−0.0482 p	D = 6.0833−0.0802 p
47–49	S = 6.0079−0.1926 p	D = 7.5561−0.2168 p

Conclusions

Considering the tables and figures alone, it may be concluded that generations 2–6 show more unquestionable positive correlation than appears in any other group of generations in the whole series. It is during these early generations that the means indicated that selection was effective. The middle period of generations taken at the face value of the correlation coefficients would not promise much success for selection, for, although there are positively correlated generations, there are also significantly negative correlations which would immedi-

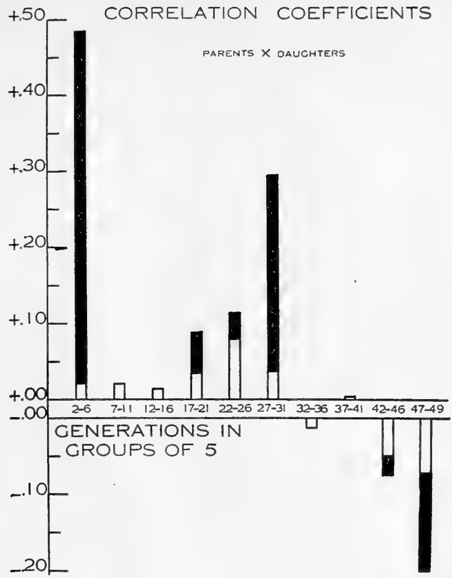


Figure 5, A

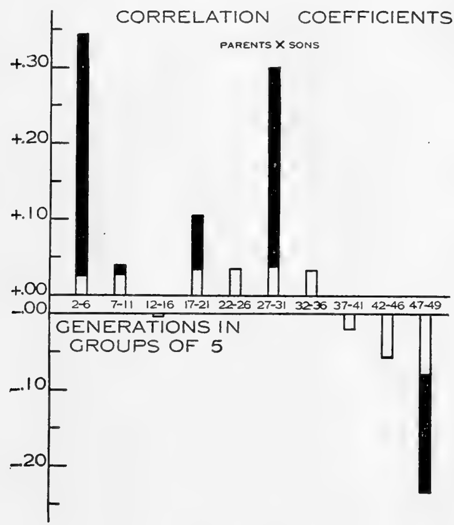


Figure 5, B

Fig. 5, *A* and *B* Correlation coefficients sons and daughters by parents, families in groups of five generations each. Plotted as in figure 1. *A*, daughters by parents; *B*, sons by parents.

ately upset any selective progress. When the underlying phenomena are considered, the possibilities for successful selection become much smaller, because the positive coefficients themselves prove to be very probably due to non-genetic causes. So it appears that the method of the correlation table has not modified the conclusions drawn from the means, namely, that after the early generations in the high-selected race, the bristle-determining germ plasm was uniform and constant; that selection in the early generations produced this uniformity by sorting out differences that existed in the original stock.

NEW DATA; SELECTION SUSPENDED

The lack of correlation shown in generations 36 to 49 has been accounted for on the basis that all the matings were made on the same day for each generation, so the environment for each set of families was as near alike as was possible to make it. There may be an objection raised to the conclusion that these uncorrelated generations indicate stable and uniform germ plasm, in that the range of the parents used was very narrow, and further, that full counts were not made of the broods. It may be argued that, even if there is no correlation shown when offspring of parents of high and very little higher grades are compared, there might still exist a degree of relationship which would be found if the whole range of parent grades were included; and, further, if the high-grade flies are more apt to appear at the beginning of a culture, the incomplete counts may have hidden a real relationship. To meet these points and to nake a final test of the conclusions already reached, four more generations were raised in large numbers without further selection.

For a long series of generations the constitution of the germ plasm in the race had been studied, in the attempt to discover the frequency of the changes that might take place therein. Only a limited number of flies could be examined in each generation, and the results were mainly drawn from a comparison of the means in different generations. The correlation method has indicated that similar conclusions are to be drawn when the germinal constitution of the different parents is judged by the

progeny they respectively produce. Instead of carrying an experiment over a long period of time and through many generations, a similar opportunity for the study of germinal phenomena is afforded by raising large numbers of families in the same generation at the same time. Indeed, the more uniform environment so secured will tend to make the germinal influence more clear. If there is no difference between the germ plasm of high- and low-grade parents, their children will all be alike, that is, there will be no tendency for high-grade parents to produce offspring of any higher grade than are produced by low parents. The correlation coefficients in this case would show no correlation at all, and it could be safely concluded that selection will not have an effect upon the racial mean. This is exactly the same reasoning as applied to the previous generations, but in this case the answer is more critical. Fifty generations of the closest inbreeding and selection must have removed all traces of heterozygosity so completely that the discovery of positive correlation in these last generations, with their environmental uniformity, would admit no alternative to the conclusion that genetic changes must have occurred during the generations in question, and that selection accordingly could occasion further advance in the means of the race.

This experiment, even standing alone, supplies sufficient data for the establishment of a general conclusion regarding the possibility of securing, through selection, a racial modification in the bristle number of this stock after the homogeniety of the race has been unquestionably established.

Experiment

Starting from one pair of flies that came from the 49th generation of continuous inbreeding and high selection, all the progeny were graded and mated with no further selection. As in all other cases, only virgin females were mated, always with their brothers, one pair of flies per bottle. The only other restriction imposed was that the two flies mated should not differ by more than one bristle.

The first two generations of this final test gave but few flies. From the flies obtained in the second of these generations (corresponding to generation 51 of the inbreeding) 22 matings were made; these produced 4364 flies that were graded. From the

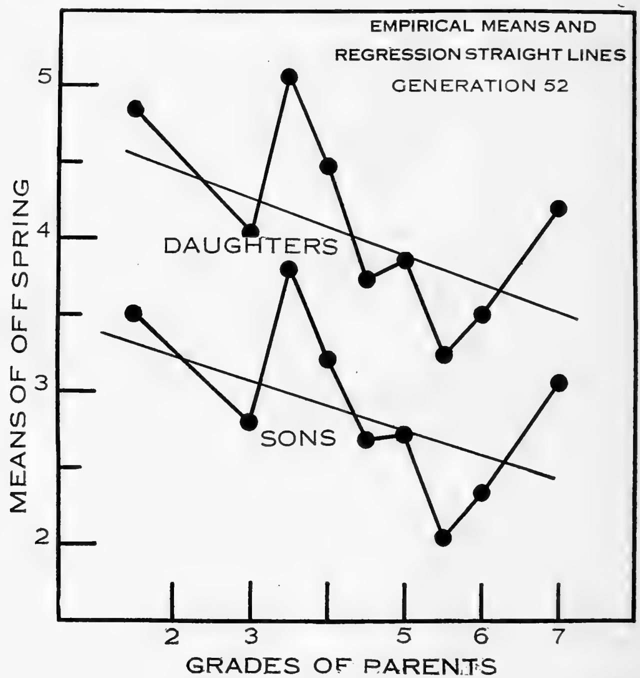


Fig. 6 Empirical means and regression straight lines in generation 52, selection suspended.

flies in the third generation, 152 matings were made; 27,133 flies from these matings were graded. Besides the common origin of these flies and their long inbred ancestry, one of the most important features of this final test was the environmental uniformity that was obtained by making large numbers of mat-

ings at the same time from the same banana culture. All the matings that produced the third generation in this series (generation 52) were made on the same day, in bottles uniformly supplied from the same banana culture. The 152 matings that gave rise to the fourth generation were all made on one of two days one week apart; those mated on the first day were trans-

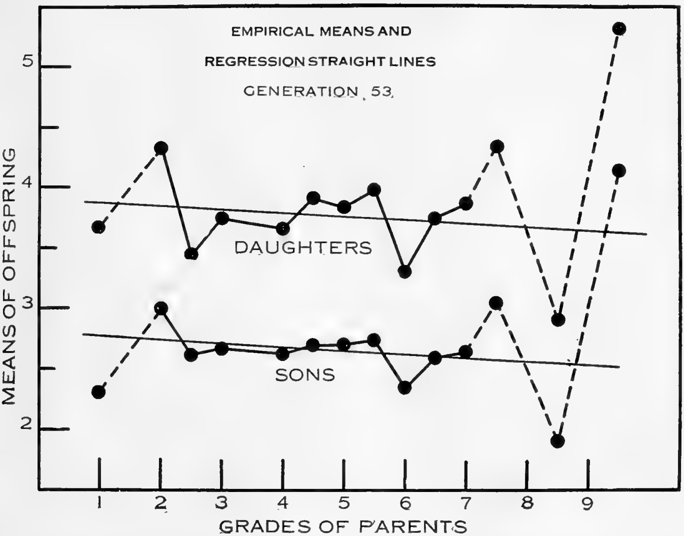


Fig. 7 Empirical means and regression straight lines in generation 53, selection suspended. The broken lines connect means from only one pair of parents.

ferred to new bottles the day the second lot of matings was started; on each day all bottles were made up from the same food.

Results

The empirical means of the offspring arranged according to the grades of the parents are shown in figures 6 and 7. As in former cases, the mean of the two parent grades has been used

where the parents were not of the same bristle grade. In generation 52 the means of the offspring fluctuate markedly; in spite of this, a straight line seems to be the best representation of their theoretical distribution. In generation 53, which includes a much larger number of parental grades, as well as nearly seven times as many offspring, the means are more uniform and more nearly approximate the theoretical straight line. The means at the extremes of the line are connected by dotted lines because they include only one fraternity each. The actual equations used in plotting the regression straight lines are as follows:

Generation 52......$S = 3.540709 - 0.0824\ P$ S = grade of sons
D = grade of daughters
Generation 53......$D = 4.823057 - 0.0932\ P$ P = grade of parents

The regression straight line make it strikingly evident that higher parents do not have higher offspring. In generation 52 the regression straight lines are strikingly inclined toward the base line, indicating a reversed relation, namely, that the higher parents produced lower offspring. In a smaller degree the same thing is true of generation 53. The correlation coefficients are as follows:

	MALES	r/E_r	FEMALES	r/E_r
Generation 52.........	-0.1436 ± 0.0266	5.30	-0.1378 ± 0.0168	8.20
Generation 53.........	-0.0271 ± 0.0069	3.92	-0.0221 ± 0.0068	3.25

When the flies are averaged in groups according to the grades of their grandparents, the lines shown in figure 8 are obtained. The high means corresponding to grandparental grade $5\frac{1}{2}$ strongly emphasize a tendency for successive points between grades 4 and $5\frac{1}{2}$ to rise. But this mean depends upon a single pair of flies and includes a smaller number of grandchildren than any other mean in this group. If this point is omitted, the remaining means assume the appearance of random distribution about the regression lines. These regression straight lines are nearly parallel with the base line; for granddaughters it is slightly descending; for grandsons it is slightly ascending. The actual regression equations are as follows:

GD = 3.8702684−0.00874 GP	GD = grade of granddaughters
	GS = grade of grandsons
GS = 2.5877662+0.00732 GP	GP = grade of grandparents

The correlation coefficients calculated from these data are:

	r	r/E_r
GD × GP	−0.0122±0.0068	1.79
GS × GP	+0.0123±0.0070	1.75

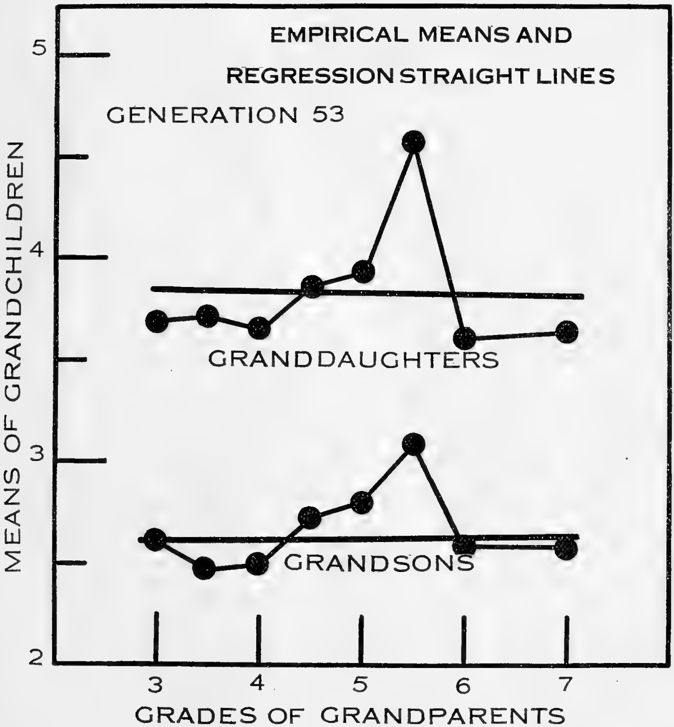


Fig. 8 Empirical means and regression straight lines for generation 53; grandparents by grandsons and granddaughters.

From all this it is evident that high-grade grandparents are not more likely to produce high-grade grandchildren than are low-grade grandparents.

Conclusion

When the germ plasm of a large number of long inbred flies with the same pedigree and the same environment is tested by breeding, it has been found that the different bristle grades are not associated with different germ plasm. This final test fully substantiates the conclusions that selection could not make further progress in this race and that the germ plasm was not changing during the time of observation.

MALES AND FEMALES

All through these experiments the males and females have been tabulated separately. The means and standard deviations of the females are regularly greater than those of the males. There is a close similarity between the direction of the fluctuations of the means of the two sexes (MacDowell, '17, p. 114, fig. 3), but there is a greater difference when the means are higher. This is so true that the graph of the differences between the means of the sexes follows closely the fluctuations of the means themselves. The correlation coefficients for the males are usually lower and less often significant than those of the females. Both sexes show the same general relationships to the parents, yet in individual generations there are differences in significance and even in sign. Such differences, as in generations 9, 17, 32, and 35, are clearly exceptions, yet they show the amounts of difference possible between random samples of flies from the same environment and the same parents.

That linkage is not a probable explanation of this sexual difference has been shown by reciprocal crosses (MacDowell, '15, p. 76, table 9, fig. 4). To make a further test for sex linkage, correlation coefficients have been calculated for sons by their mothers and by their fathers, and for daughters by their mothers and by their fathers. Since most pairs of parents were

of the same bristle grade, there were many generations in which the coefficients for the means of the parents would be the same as the coefficients for the parents taken separately. The available generations were further limited by the lack of correlation; when coefficients were too small to be significant, there would naturally be no chance to compare the different amounts of correlation when the parents were taken separately. Five generations have been used in making these tests, namely, generations 6, 27, 28, 29, and 30. The coefficients are given in table 6.

If any sex linkage is involved, mothers by sons might be expected to give higher values than mothers by daughters. The table shows that when the daughters correlated to the average of the parents, give higher coefficients than the sons similarly correlated, they also give higher coefficients than the sons, when both are correlated to the mothers and fathers separately. In other words, the same relationship holds between sons and daughters whether they are correlated to the average of their parents or to their mothers and fathers separately. Mothers and daughters are more closely correlated than mothers and sons in four of the five generations. Fathers by daughters are more closely correlated than fathers by sons in four cases. The averages of the coefficients when both relatives are females (mothers by daughters), when one relative is female (mothers by sons and fathers by daughters), and when neither relative is female (fathers by sons) are, respectively, ♀ × ♀ = 0.1685, ♀ × ♂ = 0.1103, ♂ × ♂ = 0.0998. These statements do not take into account the fact that the coefficients in generation 29 are negative; yet the agreement of these coefficients with the other cases rather strengthens the following interpretation. The coefficients indicate that no sex linkage is apparent. The females, whether mothers or daughters, raise the degree of correlation; the differences in the amounts of correlation seem to be due to the differences in the variability in the sexes, rather than to any genetic differences in the relationships of the fathers and mothers to their sons and daughters. The difference in the amount of variability in the two sexes appears to be due to some restriction of the higher bristle grades in the males. The

TABLE 6

Correlation coefficients of mothers and fathers separately by sons and daughters to test for sex linkage

GENERATION	MOTHERS BY DAUGHTERS	MOTHERS BY SONS	FATHERS BY DAUGHTERS	FATHERS BY SONS	PARENTS BY DAUGHTERS	PARENTS BY SONS
6	$+0.1564 \pm 0.0212$	$+0.0566 \pm 0.0219$	$+0.0852 \pm 0.0216$	$+0.0438 \pm 0.0220$	$+0.1438 \pm 0.0213$	$+0.0592 \pm 0.0129$
27	$+0.1867 \pm 0.0212$	$+0.0947 \pm 0.0245$	$+0.1994 \pm 0.0229$	$+0.1504 \pm 0.0241$	$+0.2013 \pm 0.0229$	$+0.1419 \pm 0.0242$
28	$+0.1238 \pm 0.0253$	$+0.1472 \pm 0.0282$	$+0.0501 \pm 0.0257$	$+0.0838 \pm 0.0286$	$+0.0908 \pm 0.0255$	$+0.1192 \pm 0.0284$
29	-0.1531 ± 0.0257	-0.0539 ± 0.0281	-0.1303 ± 0.0258	-0.1265 ± 0.0277	-0.1751 ± 0.0258	-0.1012 ± 0.0279
31	$+0.2228 \pm 0.0393$	$+0.1720 \pm 0.0430$	$+0.1137 \pm 0.0408$	$+0.0948 \pm 0.0439$	$+0.1988 \pm 0.0398$	$+0.1549 \pm 0.0432$

general make-up of the females larvae affords a more favorable set of conditions for the ontogenetic development of extra bristles.

DISCUSSION OF LITERATURE

The experiments most closely parallel to these with extra bristles are those of Castle et al. with piebald rats. A brief mention of this similarity was made in the first number of this series. In preparation for the discussion of the literature in the light of the final results, further studies of Castle's data were made; these grew to such proportions that their independent publication seemed advisable (MacDowell, '16). This discussion, both through its origin and content, should rightly appear at this point. The conclusions reached at that time seem to be substantiated by the conclusion of the subsequent discussions.

New data were presented by Castle (Castle and Wright, '16) after the writing of that paper (although published later, it was obviously written many months earlier; MacDowell, '16, footnote p. 739). The new data are quoted in full (Castle, '17) as finally breaking down the hypothesis of modifying factors (p. 113).

> I have now presented the evidence that has led me to reject the hypothesis formerly held tentatively that modifying factors were largely concerned in the changes produced in the hooded pattern of rats under repeated selection. The evidence seems to me to admit of only one consistent interpretation, that a single variable genetic factor was concerned in the original hooded race, that a changed condition of this factor was produced in the minus race, and another changed condition in the plus race, and a third appeared in the mutant race.

However, after this Little ('17) has reviewed all the rat crosses and concludes that "they offer distinct evidence of segregation." Sturtevant ('18) has not been convinced that modifying factors are eliminated by the new data; he emphasizes the need of further information in regard to the rat pedigrees and questions the crucial value of the second back-cross, so strongly stressed by Castle. The questions that Sturtevant raises concerning the second back-cross may very well lead to an explanation of the results obtained, for a third back-cross to wild of the extracted hooded rats has given such clear evidence that Castle ('19) now concludes:

This result indicated that three crosses with a third race had sufficed practically to eliminate whatever differences that had been produced in the minus and plus races respectively by long-continued selection in opposite directions. Those differences were based on residual heredity, not in changes in the gene proper.

These results favor the widely accepted view that the single gene is not subject to fluctuating variability, but is stable like a chemical compound of definite constitution and changes only similarly, by definite steps (mutations in the sense of Morgan, not of De Vries).

The five years since the review included in the first number of this series have yielded large amounts of evidence upon the selection problem. Zeleny and Matoon ('15) selected for increase and decrease in the number of facets in the bar-eyed mutant of Drosophila; three generations separated the flies into two groups with different means. The increase in the regression of the means to the parental populations is taken to indicate that selection cannot be expected to carry the bar eye all the way back to normal. Further experiments with this same character were carried on by May ('17). Using flies that had vestigial wings besides bar eyes, selections were made for five generations (2000 facet counts), with no results after the first generation; this first selection brought about a separation of the means of the two lines. Sterility in this stock led to the recommencement of the experiment using normal long-winged flies. Six generations of selection in this stock (7000 counts) effected a continuous divergence in the means of the two lines, due to the rise of the means in the high line. Return selections from the sixth generation still were successful. Unfortunately, no constants are given for the results of crossing the two lines nor are data available for their calculation. Although the author includes in the summary the statement that "the results of these experiments indicate that the hereditary differences in this race of Drosophila are due to a large number of small factors," no evidence discriminating between the theories of selection depending on separate units, and on one varying unit, is afforded.

Roberts ('18) has selected vestigial winged flies for thirty-four generations with no general advance in the means. Crossing selected inbred flies to normals changed the means, but did not

render the race susceptible to selection. This seems to be an unusual case; the general experimental result is to find some modification in the means following selection, The greater the environmental control, and accordingly the less accurately the character represents the germinal condition, the more ineffective becomes selection. In the case of the vestigial wings, it is quite possible that the strong temperature factor sufficiently upset the connection between the germinal and somatic conditions to make selection ineffective.

An interesting race of flies in which one extra dorsocentral bristle frequently appeared has been reported by Reeves ('16). All flies in the race, both the normals and those bearing extra bristles produced an excess of normal offspring as well as some with the extra bristle. The ratios range from 90:1 to 3.3:1. There appeared more extra flies from matings between extras than from matings between normals and extras, which, in turn, gave fewer extras than the matings between two normals. The sums of all the normal and extra flies from each type of mating in this race give the following ratios: four generations extra by extra, 603 normals 90 extras 6.7:1; five generations extra by normal, 1684 normals, 87 extras, 19.3:1; one generation normal by normal 335 normals, 8 extras, 41.8:1. The race was started by five flies with extra bristles being crossed with normals from the same stock; after this brother by sister matings of the above three types were variously made during five generations. The ratios in individual families were found to show a certain amount of grouping about the ratios 3:1, 15:1, 63:1. On the basis of the grouping of these ratios, the conclusion was drawn that a Mendelian explanation was possible; the higher ratios indicating that respectively two and three multiple or duplicate genes were involved. The author is quite right in saying that there is no dominance in the ordinary sense, and in so saying proves the utter impossibility of explaining the ratios in the above simple Mendelian sense. The ratios 3:1, 15:1, and 63:1 are F_2 ratios indicating the proportion of full recessives that appear after an F_1 in which all the individuals are dominants. To assume that the ratios obtained may possibly be Mendelian supposes that

dominance steps in and determines the proportion of extras that appears, then steps out when these extras, no longer recessives, are bred and give from 71.5 per cent to 95 per cent normals. However much the dominance may alternate in the parent flies, it is with peculiar consistency that the normals appear predominatingly among the progeny.

The case has obviously not been analyzed; one may suppose that the persistence of the extra flies in the race indicates some relationship between the extra bristle and the germ plasm of the race. Beyond this the data indicate that the race in question would offer excellent material for clearing up just such types of characters whose inheritance seems to be irregular and weak. The statement is made that the results agree with those of Gates ('15) and Shull ('14). Although Gates did find irregular ratios in his later rubricalyx crosses, there is no fundamental likeness between the inheritance of this extra bristle and that of Oenothera or of Bursa: in both these cases the dominance in the first generation was complete and the recessive form did not give dominants.

The introduction of linkage relations as a method of analyzing complicated genetic situations has occasioned a great advance in the solution of selection problems. However, it is only in such extensively investigated material as Drosophila that this method can be used. Since the first cases of the employment of this method (Dexter, '14, and Altenburg and Muller, see Morgan, Sturtevant Muller Bridges, '15, p. 191, where the case of truncate wings is presented) several difficult cases have been cleared up by this method. Of these, the most complicated and far reaching in its bearing, is the case of beaded wings; first investigated by Morgan, then taken up by Dexter, and finally fully cleared up by Muller ('18). Muller points out very clearly (p. 426) that the action of this character in inheritance could have been used to give strong support to the theory of factorial inconstancy, yet the linkage relations support his hypothesis of balanced lethals so strongly that the conception of factorial inconstancy is conclusively eliminated. A clear summary of this work is given by Morgan ('18, pp. 386–390).

Two investigators have used the linkage method in connection with the genetics of bristle characters, Payne ('18) and Sturtevant ('18). Payne selected for increased numbers of bristles on the scutellum; for twenty-nine generations the means seemed to respond to selection. During this time there were two periods, generations 11 to 14, 18 to 25, during which there was no advance. These are thought to be due to the attainment of homozygosis; the subsequent advances are taken to indicate the appearance of new mutations. Unfortunately, no special study was made of the environmental influences, and in their absence the fluctuations in the curve of the means can have but little weight in indicating the genetic constitution of the race (MacDowell, '17, p. 139).

The linkage relationships indicate that there is a bristle factor in the sex chromosome and probably one in the third chromosome; but the relation of these factors to the advance of the selected means has not been satisfactorily demonstrated. The suggestion that only one factor was concerned at the beginning of the experiment does not find support in the ratios (p. 7). It is to be regretted that neither of these factors was isolated and its Mendelian nature proved by crosses.

Payne mentions two races that were separated by selection from a low-grade mutant; the complete factorial analysis of these races in connection with the high-selected race should yield interesting results. Having shown that extra bristles in his flies depend upon more than one factor, Payne applied the test of crossing the selected race repeatedly to normals; if multiple factors are involved, the successive crosses should show increasing regression. However, this result was not found. In view of the uncertainty as to the environmental influence and the relationship and generic homogeneity of the wild males used in the crosses, this result can hardly invalidate the method. This is the test that Castle has accepted as crucial and has occasioned his final adoption of the mutliple-factor hypothesis.

Sturtevant has analyzed the effect of selecting the variable numbers of dorsocentral and scutellar bristles that appear associated with the mutation called dichaet; besides influencing the

bristle number, this mutation causes a spreading of the wings, a character more diagnostic than the modified bristle number. Unlike either of Payne's factors, dichaet gives simple ratios that prove it to be primarily dependent upon a single gene; its linkage shows it to lie in the third chromosome. Modifiers of the bristle numbers are located in the second and third chromosome; in each of these chromosomes a modifier was shown to cross over. Selection separated out two races; crossing these two races together resulted in increased variability in the F_2; return selection was successful when started from the early generations of the advance, and usually unsuccessful when started from the later generations. The influence of the environment was recognized as causing a significant part of the variability observed. Small flies had fewer bristles, both small flies and fewer bristles appeared when food conditions were bad, as at the end of a culture.

A searching discussion based on the following penetrating questions concludes Sturtevant's paper:

> Does selection use germinal differences that are already present, or differences that arise during the experiment, or both?
>
> In the case it uses new differences, does it cause them to occur more frequently, and does it influence their direction?
>
> Are differences, already present or arising de novo more likely to occur in the locus of the gene under observation, or in other loci? (p. 36).

After citing twelve cases formerly cited as evidence favoring the theory of genetic contamination, he concludes that such an hypothesis is unproved, unnecessary and confronted with some directly contrary evidence. The first paragraph of the general conclusions should be read in full:

> That many characters may be influenced by more than one pair of genes has long been recognized, and this is the essence of the multiple-factor view. That genes exist that require the action of other genes before they produce visible effects has also been long known. Furthermore, that there are genes that produce very slight visible effects is now another commonplace. Given these three facts and the hypothesis (which is supported by much specific evidence) that most races are heterozygous for many such genes is all that is required to

complete the conception that is held by most adherents of the view that multiple factors or modifying genes are responsible for the results of selection (p. 51).

Among the authors who do not accept the substance of the multiple-factor theory may be mentioned Goldschmidt and Sumner. Goldschmidt ('18) gives a brief outline of experiments with gyspy-moths. His results are said to agree closely with those of Castle. Different races are characterized by different amounts of pigment in the larval stages; crosses between any of these races always give simple 3 : 1 ratios. In certain races the degree of pigmentation shifts with the successive stages of larval development; a time factor introduced that is inherited independently of the color factor and determines the degree of pigmentation that is finally attained. The interpretation is based on the hypothesis than an extensive series of multiple allelomorphs is involved in distinguishing the different races. These really are different quantities of a substance that determines the velocity of the reaction of pigment formation. Given a constant developmental factor and the quantity of this substance will always determine the degree of pigmentation reached. In support of the idea that even within the same race the amount of this substance may vary and so yield to selection, the results of selecting in the F_1 of a cross are cited. Although the F_2 gave the 3 : 1 ratio as always, the grades of the offspring from different F_1 grades were correspondingly different. Hence there were variations in the substance in the parent races. The full report of Goldschmidt's experiments will present much interesting and valuable data, but, if the phenomena shown by his moths so closely resemble those shown by the hooded rats, one wonders if the same interpretation will not be required by both experiments. Either the Goldschmidt theories will have to be made to fit the mass of data that leans in the opposite direction, or else the full data will be found to accord with the hypothesis that the majority of experiments seem to demand.

From the behavior of different geographical races of Peromiscus when the environments were interchanged and when raised in cages in the same environment, and when crossed, Sumner ('18)

concludes that such racial differences are inherited. Mutations were found that were believed to represent a different kind of inheritance.

> I insist that the burden of the proof rests on those who contend that these two types of variation and inheritance are reducible to a single category, that of discontinuity. Anything like a proof of this contention appears to be lacking (p. 452).

However carefully performed and interesting in themselves are these experiments of Sumner, they professedly are not critical and stand as opposition mainly on account of their general appearance of blending, which is so interpreted with far less difficult experimenting than is required by the current theory. It is obvious that objections based on evidence of this sort do not have much weight in combating the conclusions supported by the array of analytical selection experiments.

SUMMARY

1. The early generations of a race of Drosophila that was continuously selected for increased bristle numbers during forty-nine generations show higher correlation coefficients than any others.

2. With the greatest uniformity of the environment, the last thirteen generations show no correlation at all.

3. A final test of the germ plasm of the selected race was made by raising four more generations without selection. In generations 52 and 53, 31,000 bristle counts indicated that the higher-grade parents did not produce higher-grade offspring; the correlation was negative for parents by sons and by daughters as well as for grandparents by grandsons and granddaughters.

4. No evidence of sex linkage is found by comparing the coefficients of mothers and fathers correlated separately with their sons and daughters. The difference between the sexes is interpreted as due to the general developmental conditions initiated by the sex chromosomes, rather than to the linkage of a specific gene.

5. The correlation method supports the general conclusions previously drawn; there were genetic differences present among the original flies with extra bristles; these were entirely independent of the main factor that occasioned the monohybrid ratios in crosses; selection propagated the more homozygous flies, so that a race with uniform germ plasm was soon secured. There is no indication that any genetic change occurred during the course of the experiments.

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Resumen por la autora, Minna E. Jewell.
Universidad de Illinois.

Los efectos del hidrógeno en concentración iónica y el contenido de oxígeno del agua sobre la regeneración y metabolismo de los renacuajos.

La autora ha estudiado la marcha de la regeneración y la de la producción de anhidrido carbónico en los renacuajos bajo diversas condiciones de concentración del ión hidrógeno, temperatura y contenido de oxígeno del augua. La concentración optima del ión hidrógeno es aproximadamente la de neutralidad. Las variaciones de este óptimo en cualquiera de las dos direcciones, así como las temperaturas bahas o el escaso contenido de oxígeno del agua, disminuyen progresivamente la marcha y cantidad total de la regeneración. La disminución de la regeneración bajo condiciones no favorables se debe a dos factores: al efecto directo sobre el tejido en vías de regeneración y al efecto sobre el conjunto del animal. El primer factor es independiente del tamaño de los renacuajos, mientras que el segundo aumenta a medida que disminuye el tamaño de estos. La disminución de la producción de anhidrido carbónico en ácidos y el aumento en las bases susministran curvas semejantes a las de la disminución de la regeneración en condiciones semejantes. La autora incluye en el trabajo curvas y cuadros que indican la regeneración y producción de anhidrido carbónico en renacuajos bajo las diversas condiciones ambientes estudiadas, discutiendo la significación teórica de los datos obtenidos.

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Carnegie Institution of Washington

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THE EFFECTS OF HYDROGEN ION CONCENTRATION AND OXYGEN CONTENT OF WATER UPON REGENERATION AND METABOLISM OF TADPOLES[1]

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TWENTY-FOUR FIGURES

CONTENTS.

INTRODUCTION

The direct influence of temperature upon the rate of development has long been recognized, and has recently received considerable attention, especially from the economic entomologists who are working upon the relation of weather conditions to the time of emergence of insect pests. That other environmental factors may similarly affect development is also recognized, but as yet the literature is meager.

[1] Contribution from the Zoological Laboratories, University of Illinois, no. 153.

Probably no single environmental factor is of greater importance to aquatic animals than the chemical reaction (hydrogen ion concentration) of the water. Shelford and Powers ('15) have shown that marine fishes are extremely sensitive to slight variations in the hydrogen ion concentration of the water, and Wells ('15) has shown the same to be true of fresh-water fishes.

The first study of the effect of hydrogen ion concentration upon the rate of development is that of Loeb ('98). He compared the development of eggs of the sea-urchin in normal sea-water with those in sea-water to which 2 cc. 0.1N NaOH per 100 cc. had been added. At first no difference was noted, but after the thirty-two- to sixty-four-cell stages it was evident that the eggs in the alkaline solution had developed more rapidly. The embryos in normal water were still blastulae when those in alkaline water were plutei, and at the end of forty-eight hours, though all were plutei, the ones in alkaline water were larger, and further developed. One, two, or three cc. 0.1N HCl per 100 cc. was found to retard development.

Working upon the eggs of Fundulus in fresh water, Loeb found that 4 cc. 0.1N NaOH per 100 cc. caused them to hatch faster. More than 6 cc. retarded development; 12 cc. allowed only a few of the eggs to hatch, while 15 cc. resulted in failure of any to hatch. Two cc. of 0.1N HCl killed all of the eggs, while 1 cc. killed most of them. Loeb attributes these results to an increase of oxidation by bases and a decrease by acids.

Later Loeb ('14) decided that the acceleration of development of the sea-urchin eggs in water to which NaOH had been added did not appear until the thirty-two- to sixty-four-cell stage because at first the concentration was too high, but after a few hours the beneficial effects appeared as the result of neutralizing the acids produced by the embryos during development. These conclusions were based upon a comparison of the rate of regeneration and growth of tubularians in a solution of NaCl, KCl, $CaCl_2$ and $MgCl_2$ in the proportions in which they occur in sea-water, and in the same solution to which NaOH, $NaHCO_3$, or Na_2HPO_4 had been added. Although the growth was invariably better when a base had been added, as the effects of the

weaker bases were better than those of NaOH, Loeb concluded that the results were not due to the free OH ions, but to the ability of the base to neutralize the acids produced by the tubularian.

Loeb and Wasteneys ('13 a and b) have shown that bases increase the rate of oxygen consumption both in the fertilized and unfertilized eggs of Strongylocentrotus purpuratus, but that in fertilized eggs this increase in oxygen consumption is accomplished only by concentrations which cause a suppression of the phenomena of development.

Moore, Roaf and Whitley ('05) worked upon the effects of alkalies and acids and of alkaline and acid salts upon growth and cell division in the fertilized eggs of Echinus esculentus. The bases used were NaOH, KOH, $Ca(OH)_2$, and NH_4OH. The acids were HCl, CH_3COOH, and CO_2, and the salts Na_2CO_3, $NaHCO_3$, Na_2HPO_4, and NaH_2PO_4. These workers found that bases, except NH_4OH, give acceleration of development in low concentrations, while higher concentrations check development, and finally kill. Acids all inhibit. The primary factors affecting the rate of growth appear to be the variations in concentration of hydrogen and hydroxyl ions. Thus all of the caustic alkalies are of approximately equal power, and there is little or no action of the cation. But in the case of the phosphates, where the hydrogen-hydroxyl ion concentrations are comparatively low. there seems in addition to be a specific factor. The extreme limits of variation of hydrogen and hydroxyl ion concentrations within which growth is possible are shown to be very narrow, 0.0015N caustic alkali or 0.001N acid practically stopping all development. Whitley ('05) showed the same relationship between hydrogen-hydroxyl ion concentration and development to hold for eggs of Pleuronectes platessa. He gave three reasons why alkali is less harmful than acid:

1. Alkali added to sea-water is immediately thrown out as insoluble hydrates or carbonates.

2. Alkali is constantly used up to neutralize CO_2 produced by the animal.

3. Loeb has shown a low quality of alkali increases the permeability to O_2. This may increase the resistance of the egg.

Finally, Grace Medes ('18) has shown that changes in the composition of the sea-water by concentration, dilution, or addition of acids, bases, or salts in non-lethal concentrations cause a retardation of development of the eggs of Arcabia punctulata.

Excepting for the experiments of Loeb in 1898 on Fundulus eggs, nearly all of the work so far done on the effects of bases and acids on development has been done upon marine animals. Many of the most serious difficulties in such investigations arise as the result of the chemical composition of sea-water, and may be largely eliminated by the use of fresh-water forms. Thus Haas ('16) has shown that the addition of hydroxides to sea-water results in a proportionate increase in OH ion concentration only after all of the Ca and Mg have been precipitated out in the form of their basic carbonates. This discovery is of twofold importance, for it means, in the first place, that much of the base added to the sea-water is immediately thrown out, and, in the second place, a disturbance of the balance between the salts of Na, K, Ca, and Mg, which Loeb ('03) has shown is exceedingly harmful to marine animals.

In working with acids, too, the use of sea-water presents certain difficulties, for any strong acid added to sea-water will immediately form salts with the liberation of CO_2. Hence no comparison of the effects of the various mineral acids is possible, as the acidity existing in the sea-water is due to H_2CO_3. For this reason, and also to see whether fresh-water animals are adjusted to a different H ion optimum than marine organisms, the author at first wished to undertake a study of the effects of bases and acids upon development and growth in some fresh-water form. Since, however, there were no eggs or young larvae available at the time of year when this investigation was commenced, it was decided to use regeneration which in many ways resembles original growth. This seemed further desirable because no such studies of regeneration have as yet been made, and it is probable that comparative studies of regeneration and growth under varying environmental conditions may throw much light upon the extent of the similarity or dissimilarity of

the two processes. What is true of the effects of hydrogen ion concentration is also true of the effects of oxygen on regeneration. As yet no experimental studies are known to have been made on regeneration in varying concentrations of oxygen. As rapid oxygen metabolism and rapid growth are often associated, the carbon-dioxide production experiments were undertaken to see whether any correlation could be traced between the rate of regeneration of a part and the oxygen metabolism of the animal as a whole, and whether substances stimulating to oxygen metabolism are, as Whitley supposed, beneficial to the organism. In this work the author has been concerned only with the extrinsic factors of regeneration. The intrinsic factors have been considered only as they arose as necessary corollaries to the work on environmental effects.

Throughout this paper the word 'regeneration' has been used for those processes of cell multiplication and growth which result in the increase of size of the new part. The author is fully aware that the general term 'regeneration' is commonly used to include three processes: dedifferentiation, growth, and differentiation. In order, however, to avoid the use of the word 'growth,' which would lead to confusion of the processes under experiment with the growth of the organism as a whole, and further to avoid the repeated use of long explanatory clauses, the simple word 'regeneration' has been used in this restricted sense.

MATERIALS

The tadpoles studied were larvae of Rana clamata (Dau) collected from a spring-fed marsh near Muncie, Illinois. Three collection trips were made, dated October 9, 1917, March 5 and 24, 1918. This supply was supplemented during the middle of the winter by a few tadpoles purchased from a local dealer. All tadpoles were identified by means of key to larval amphibia, Wright ('14). The stock was kept in a greenhouse in an alberine tank supplied with running water to a depth of about 6 inches. The food supplied was filamentous green algae. All tadpoles were kept fasting during the experiments.

Measurements of regeneration were made by means of a caliper and a metal metric ruler, obtained from Spencer Lens Company. The tail was the only organ experimented upon. In making averages there was no elimination of individuals, except in one or two instances, and there note is made of the fact, and the aberrant individual included in the table.

APPARATUS AND METHODS

Oxygen-free water was obtained from the water-boiling apparatus in the vivarium at the University of Illinois, which has been described by Shelford ('18). This apparatus consists primarily of a boiler in which the water is boiled by means of high-pressure steam until practically all of the gases are driven out, and from which it passes into a tank covered by a hood where boiling is continued until the water is freed of gases. The flow of water into the boiler is regulated by a float-cock. The oxygen-free water is withdrawn from the hooded tank, and passes through cooling pipes in the room below where it is used. As it comes from the pipes it contains no oxygen (Winkler method), and is strongly alkaline, due to the CO_2 having been expelled by long boiling, and much of the bicarbonate having been converted into carbonates. A series of different concentrations of oxygen were obtained by siphoning the water through a series of half-gallon mason jars (diagram 1), through alternate ones of which air was bubbled. The water from the last of these was siphoned into a 5-inch beaker, so that the level of the water in the jars could not fall below 5 inches. It was found by titration that the oxygen concentration varied greatly in the different parts of a jar through which a stream of air was passing, but was relatively constant except at the surface in a jar through which the water was being siphoned but no air was passing; consequently the jars containing experimental animals were alternated with those which were being aerated, with the result that each experimental jar contained a little more oxygen than the one preceding.

The flow of air through the water was regulated by passing it through capillary tubes drawn to a fine point, and the air pressure was kept relatively constant by means of a mercury manometer made by bending a U at one end of a 5-foot glass tube, and attaching it at the end of the air line. Mercury was then poured into the tube until a pressure was reached at which air would bubble through the jars at the desired rate.

Daily O_2 determinations were made on water from each jar by the Winkler method. Samples for these determinations were

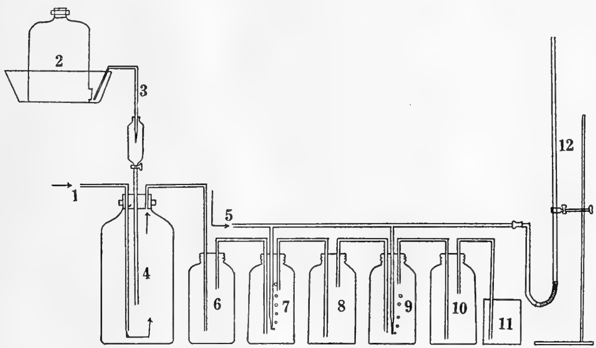


Diagram 1 Apparatus for securing a series of low concentrations of oxygen. *1*, inlet for oxygen-free water; *2*, aspirator bottle containing acid; *3*, syphon; *4*, mixing bottle; *5*, inlet of air line; *6*, *8* and *10*, experiment jars; *7* and *9*, aerating jars; *11*, beaker; *12*, manometer containing mercury.

collected by introducing a Powers' sampling bottle (Powers, '18) between two jars, and allowing the water to siphon through it for fifteen minutes.

As has been mentioned above, the oxygen-free water from the boiler is strongly alkaline. Where neutral water was desired, this was remedied by means of the acidulator (upper left of figure 1) which consists of a 12-liter aspirator bottle placed in a large earthenware milk pan, with the upper opening securely stoppered. The acid in the pan stands at the level of the top of the lower opening in the bottle, thus giving a constant pressure in the

siphon which is drawn to a capillary tube at the end. The flow of the acid is regulated by the size of the capillary tube. The acid drops from the siphon into the separatory funnel, through which it passes to the bottom of the 10-liter mixing bottle, where it is thoroughly mixed with the water before entering the experimental jars: 0.1N H_2SO_4 was used. The acidity of the water could be regulated by counting the number of drops of acid per minute and regulating the flow of water through the mixing bottle accordingly.

The distilled water

In some preliminary experiments ordinary distilled water obtained from the Department of Chemistry was used. This water is condensed in a copper condenser, and passes through block-tin pipes. It was found not only to be very toxic to tadpoles, but to vary in its effect from day to day, so that tadpoles might live several days in water taken from one bottle, and die in a few hours in water taken from another. The same water redistilled in glass supported the life of tadpoles of Bufo lentiginosus Le Conte through metamorphosis. These results are in accord with the findings of Bullot ('14), and Powers ('18 b), who attributes the toxic effect of ordinary distilled water to traces of colloidal copper taken up from the condenser. Powers has shown that a goldfish may live fifty-one days in water redistilled in glass, but succumbs quickly if the minutest trace of colloidal copper be added to the water.

The water for all of the experiments was condensed in a 6-foot Jena glass condenser attached to the second tank of the water-boiling apparatus mentioned above. It was then aerated for twenty-four hours by a stream of air previously passed through sulphuric acid and calcium hydroxide wash bottles. This distilled water was neutral to rosalic acid and neutral red. An analysis of the water kindly made by the analyst of the State Water Survey was as follows:

Residue on evaporation:	
Total solids	38 to 42 parts per million
Alkilinity as calcium carbonate:	
Methyl orange	0 to 0 parts per million
Chlorides:	
As sodium chloride	0 to 0 parts per million
Ammonia nitrogen	4 to 7 parts per million
Albuminoid nitrogen	0.060 to 0.102 parts per million

A common shiner (Notropus blennius) lived in this water, changed weekly, from October 18, 1917, to February 17, 1918, or a total of one hundred twenty-two days. As, during this time, the fish decreased notably in size, its death might be attributed to starvation rather than to the toxicity of the water. Powers (unpublished) has shown that two goldfishes lived in this water ninety-five and ninety-nine days respectively, whereas in ordinary distilled water the length of life was three hundred fifty-two and five hundred and ninety-seven minutes.

All chemicals used were either Mercks' or Baker's analyzed chemicals. The acids and bases were standardized at 0.01N, except H_3PO_4, Na_2CO_3, and $NaHCO_3$, which were 0.01 mol.

For the determination of CO_2 production, 1-pint clamp-top fruit jars were used. The capacity of one of these jars is about 500 cc. Four hundred cc. of water or the solution under investigation was put in a jar, then a test-tube containing 10 cc. 0.02N $Ba(OH)_2$ was inserted so that the top of the test-tube stood above the level of the water, the rubber ring was adjusted, the tadpole put in, and the lid clamped down. A stronger solution of $Ba(OH)_2$ was used where acid had been added to the water or where more CO_2 was expected. To correct for the CO_2 in the water and in the air above, a control jar was prepared at the same time and in precisely the same manner, except that no tadpole was put in, and placed beside the experiment. At intervals, usually 24 hours, the $Ba(OH)_2$ was mixed with the water by inverting and rotating the jar, the tadpole was quickly removed, and the excess of $Ba(OH)_2$ titrated with 0.01N H_2SO_4, using phenolphthalein as indicator. The difference in the amount of acid required to neutralize the experiment and the control was recorded as the amount of CO_2 (as 0.01N H_2CO_3)

produced by the tadpole during the given time. This method of CO_2 determination is based upon the assumption that all of the acid produced by the organism is CO_2, or that other acids, if produced, are in negligible quantities.

The hydrogen ion concentration was determined by the method of McClendon ('16). The solution to be tested was placed in a test-tube 1-cm. bore, and 0.1 cc. of the indicator solution added. It was then compared with the color chart by looking down into the tube against a white background. The indicators used were butter yellow (dimethyl amido azobenzene), methyl orange, 4 Br-phenol-S-phthalein (brom phenol blue), methyl red, paranitrophenol, neutral red, 2 Br-thymol-S-phthalein (thymol blue), phenolphthalein and thymolphthalein. Solutions of methyl violet and tropaeolin 00 (orange IV) were also prepared, but none of the solutions used were so acid as to come in their range. On the other hand, the basic solutions went beyond the range of the color chart, so the hydrogen ion concentrations of only the more dilute solutions could be determined.

This method, as applied, is at best only approximate, for, in the first place, the comparison of a transparent solution with an opaque paper color chart is difficult, and gives great scope for personal equation, and, in the second place, where the solution employed caused extensive injury to the animal it is probable that amino acids and other organic compounds from the degeneration tissues were set free in the solution. Such substances may affect the delicacy of the indicator.

PRESENTATION OF DATA

Before beginning the experiments on regeneration, a test was made to determine approximately the concentration to use. 0.01 and 0.001N solutions of each of the bases and acids to be tested (except Na_2CO_3 and H_3PO_4 0.01 and 0.001 mol.) were prepared, and a tadpole put into 1 liter of each. The length of life was noted as follows:

BASE		KOH	NaOH	$Ba(OH)_2$	$Ca(OH)_2$	NH_4OH	Na_2CO_3
0.01 N	Length of life in minutes.	50	60	65	60	35	
0.001 N	Length of life in days....	31	5	$1\frac{1}{2}$	30	$\frac{1}{6}$	6

ACID		HNO_3	HCl	H_2SO_4	H_3PO_4	CH_3COOH
0.01 N	Length of life in minutes	70	120	125	135	125
0.001 N	Length of life in days	1+	2–	5	8	7

Two controls were run; one distilled water from the metal still (this water was used in making up the solutions) and the other, to eliminate the possibility of osmotic pressure having produced the effect, a solution consisting of 37 parts 0.01 mol. KH_2PO_4 and 63 parts 0.01 mol. Na_2HPO_2, which is the neutrality buffer solution of Levy, Rowntree and Mariott ('15) diluted to 0.01 mol. In the former the tadpoles lived five days, in the latter thirty-eight days.

It will be noted with regard to the bases that in both concentrations the tadpoles in NH_4OH, the weakest base, died first. For this reason, as the specific toxicities of the various substances were not within the scope of this investigation, NH_4OH was not used in subsequent experiments. The difference in length of life of tadpoles in 0.001 N. KOH and NaOH (thirty-one and five days) is not so significant as it at first appears. Both solutions produced very serious effects upon the tadpoles during the first few days. The skin became white, the margins of the tail degenerated, and both specimens appeared on the point of death. A test of the water made the fourth day showed that it was then practically neutral. From this time on the tadpole in KOH gradually recovered, but its companion in NaOH succumbed. It would seem, however, and subsequent experiments tend to show, that the considerable difference in length of life was caused by a slight difference in the resistance of the two individuals. Similarly the death of the tadpole in Na_2CO_3 after six days was not regarded as showing any specific toxicity of that substance, although the death of the tadpole in $Ba(OH)_2$ after thirty-six

hours was suggestive of poisoning. Acetic acid (CH_3COOH) was also excluded from subsequent experiments pending further work upon the possible toxic effect of the organic radical.

TABLE 1

Regeneration in KOH. *Length of tadpoles 53 to 59 mm.*

0.01 N KOH IN 200 cc. H_2O	LENGTH IN MILLIMETERS										
	Re-moved		REGENERATEDS								
			5th day	8th day	11th day	15th day	20th day	23d day	26th day	28th day	30th day
cc.											
0	14.3	mm.	1.2	2.2	3.4	4.6	5.6	5.8	6.4	6.2	6.2
		%	8.4	15.4	23.8	32.0	39.0	40.5	45.0	43.5	43.5
0.25	12.0	mm.	1.3	2.5	3.1	4.7	5.1	5.4	5.8	5.8	5.8
		%	10.8	20.8	26.0	38.0	42.5	45.0	47.0	47.0	47.0
0.5	12.5	mm.	1.1	2.5	3.3	4.3	4.8	5.0	5.5	5.5	5.2
		%	8.8	20.0	26.0	34.4	38.0	40.0	44.0	44.0	42.0
2.0	12.9	mm.	1.1	2.2	3.2	4.3	5.1	5.3	5.3	5.4	5.3
		%	8.5	17.0	25.0	33.3	39.5	41.0	41.0	41.0	41.0
5.0	13.3	mm.	1.0	2.6	3.7	4.6	4.6	4.7	4.8	5.1	5.1
		%	7.5	19.5	28.0	34.6	34.6	35.3	36.0	38.4	38.4
10.0	12.0	mm.	0.8	2.0	3.4	4.4	4.0	4.0	4.0	4.0	4.0
		%	6.6	16.8	28.3	36.6	33.3	33.3	33.3	33.3	33.3
15.0	13.8	mm.	0.8	1.3	2.8	3.5	3.6	3.8	Dead		
		%	5.8	9.4	20.0	25.0	26.0	27.5			
20.0	12.9	mm.	0.2	1.1	2.0	3.1	3.3	Dead			
		%	1.5	8.5	15.5	24.0	25.6				
25.0	12.1	Died without regeneration									

Total length and readings for ninth, thirteenth, and eighteenth days omitted.

Regeneration in bases

Tables 1 and 2 give the regeneration of individual tadpoles, large and small, respectively, in KOH. Figures 1 and 2 are curves of growth drawn from the same data. These experiments

were carried out in finger bowls each containing two tadpoles (one of each size) in the amount of base indicated in the table made up to 200 cc. with distilled water. The solutions were changed daily throughout the experiments. In plotting the

TABLE 2

Regeneration in KOH. *Length of tadpoles 24 to 28 mm.*

0.01 N KOH IN 200 cc. H_2O	LENGTH IN MILLIMETERS										
	Re-moved	Regenerated									
			5th day	8th day	11th day	15th day	18th day	20th day	23d day	26th day	28th day
cc.											
0	5.3	mm.	1.2	2.3	2.9	3.2	3.2	3.3	3.3	3.2	3.2
		%	23.0	44.0	55.0	60.0	60.0	62.0	62.0	60.0	60.0
0.5	6.1	mm.	1.3	2.0	2.6	2.9	2.9	2.9	3.0	3.0	3.0
		%	21.0	33.0	42.6	48.0	48.0	48.0	49.0	49.0	49.0
1.0	6.1	mm.	1.4	2.4	3.0	3.1	3.1	3.1	3.2	3.2	3.2
		%	23.0	39.0	49.0	51.0	51.0	51.0	52.0	52.0	52.0
2.0	5.9	mm.	1.1	2.1	2.6	2.7	2.7	2.7	3.0	3.0	Dead
		%	20.4	37.0	44.0	46.0	46.0	46.0	51.0	51.0	
5.0	5.2	mm.	1.2	2.0	2.5	2.7	2.7	2.7	2.7	Dead	
		%	23.0	38.5	48.0	52.0	52.0	52.0	52.0		
10.0	5.5	mm.	1.0	1.5	2.0	2.2	2.2	Dead			
		%	18.2	27.3	36.4	40.0	40.0				
15.0	4.8	mm.	0.6	0.9	1.3	1.8	1.0	Dead			
		%	12.5	18.7	27.0	27.5	20.8				
20.0	6.0	Died without regeneration.									

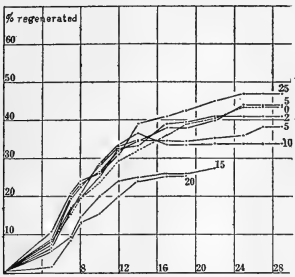
Total length and readings for ninth and thirteenth days omitted.

curves the per cent regenerated, taking the amount removed as 100 per cent, was used instead of the length actually regenerated because, as Zeleny ('16) has shown, "Within wide limits the length regenerated in the tail of an amphibian larva is directly proportional to the length removed." Practically identical

results were obtained in a parallel series of experiments in which the base used was $Ca(OH)_2$.

The large range of low concentrations was used to see if any acceleration to regeneration might be produced by low concentrations, such as was found for the development of sea-urchin eggs by Loeb ('98) and Moore, Roaf and Whitley ('05). As the growth curves show, no such acceleration appeared. In fact, the addition of bases produces no appreciable effect until 15 cc. has been added, giving a 0.0075 normal base, in which concentration a marked retardation of regeneration appears in both KOH and $Ca(OH)_2$. A comparison of tables 1 and 2 brings out another point. The larger tadpoles (table 1) show a marked retardation of regeneration in 15 and 20 cc. of the base. The smaller tadpoles (table 2), although killed much sooner by these higher concentrations, show no greater retardation of regeneration in the concentrations next lower—10 and 15 cc.—than do the larger ones. This subject of the effect of size upon inhibition of regeneration will be more fully discussed later.

The next series of experiments (fig. 5) was run in order to determine whether the effect of bases upon regeneration is due to the action of the hydroxyl ions or whether the cations or osmotic pressure play an important part. The experiments were carried on in finger bowls each containing four tadpoles. The water was changed alternate days. Although the eighty tadpoles used were of as nearly the same size as could be obtained, they were first divided into four lots, each lot containing tadpoles apparently identical in size. One tadpole from each lot was then put into each of the solutions. This method for obtaining uniformity of size was used in all succeeding experiments where a large number of animals were involved. The first, seventh, and fifteenth dishes were distilled-water controls. In figure 5 the 'O' line, or curve of growth of the controls, is plotted from the average of these three. This figure shows a progressive and rapid decrease in regeneration in increasing concentration of Na_2CO_3. The tadpoles in 60 and 75 cc. died in four and three days, respectively, without having undergone any regeneration. There is no marked decrease in regeneration in increasing con-



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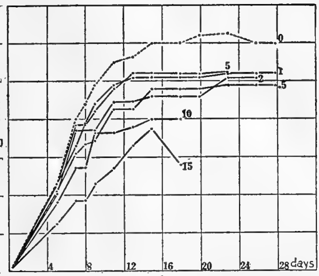

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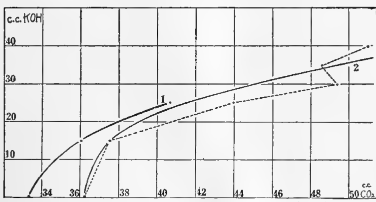

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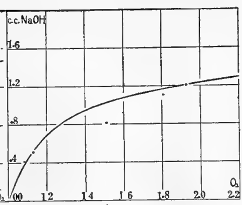

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Fig. 1 Graph showing the regeneration of large tadpoles in increasing concentrations of KOH. Each line represents the regeneration of one tadpole. *Dotted line,* control; *ordinate,* per cent regenerated; *abscissa,* time in days; *numbers at ends of curves,* cc. 0.01N KOH in 200 cc. of solution. Note decrease in rate of regeneration and total amount regenerated in higher concentrations. Compare with figure 2 which represents regeneration of small tadpoles in corresponding solutions. From data of table 1.

Fig. 2 Graph showing the regeneration of small tadpoles in increasing concentrations of KOH. Plotted as figure 1. *Dotted line,* control. A comparison of this figure with figure 1 shows the relatively more rapid and more complete regeneration characteristic of smaller tadpoles. Note also that except in case of the small tadpoles in 20 cc. of KOH, which died without regeneration, the relative effect of the higher concentrations is about the same for both sizes. From data of table 2.

Fig. 3 Graph showing daily CO_2 production of tadpoles in increasing concentrations of KOH. *Ordinate,* concentration as cc. 0.01N KOH in 400 cc. of solution; *abscissa,* CO_2 produced as cc. 0.01N H_2CO_3; *curve 1,* average CO_2 production for twenty-four days; *curve 2,* CO_2 production of the first day. A comparison with figures 1 and 2 shows that the same concentrations of KOH which increase CO_2 production inhibit regeneration. From data of table 3.

Fig. 4 Graph showing the oxygen consumption of fertilized eggs of Strongylocentrotus purpuratus in increasing concentrations of NaOH. *Ordinate,* concentration as cc. 0.1N NaOH in 50 cc. of the solution; *abscissa,* coefficient of oxygen consumed as compared to neutral controls. Drawn from the data of Loeb and Wasteneys ('13 b). This curve, which represents oxygen consumption, is essentially similar to figure 3 which represents CO_2 production in a basic medium.

centrations of $NaHCO_3$ until a 0.006 mol. solution (120 cc. 0.01 mol. $NaHCO_3$ in 200 cc.), is reached.

In the dilutions used (0.00075 to 0.00225 mol. Na_2CO_3 and 0.00075 to 0.006 mol. $NaHCO_3$) the degree of ionization of the salts would be very great, since ionization becomes complete as dilution becomes infinite. If we assume complete ionization, the number of Na ions would be the same in a given solution of Na_2CO_3 as in a solution containing twice as many molecules of $NaHCO_3$, while the osmotic pressure of equimolecular solutions of the two salts would be the same. If, then, the effect on regeneration is due to Na ions, the same retardation should be produced by 45 cc. Na_2CO_3, 30 cc. each Na_2CO_3 and $NaHCO_3$, or 90 cc. $NaHCO_3$. An examination of figure 5 shows this is not the case, and that it would, in fact, be necessary to assume twice as great ionization for Na_2CO_3 as for $NaHCO_3$ in order to explain the physiological effects in terms of the Na ions. Considering the dilution, so great a difference in ionization is highly improbable. On the other hand, it is impossible to attribute the results obtained to osmotic pressure, for even if the Na_2CO_3 is assumed to be completely ionized, and the $NaHCO_3$ completely undissociated, the effect of Na_2CO_3 would still be proportionately too great, as the depression of regeneration caused by 30 cc. Na_2CO_3 is considerable, while 90 cc. $NaHCO_3$ produces no appreciable effect. In view of these facts, and in view of the further fact that the weakest solutions used in these experiments are comparable as to osmotic pressure and number of metallic ions to the strongest solutions of hydroxides employed, it is safe to assume that the important factor in the retardation of growth produced by the metallic hydroxides is the OH ion.

Carbon-dioxide production and regeneration in bases

As basic media are generally known to increase oxygen metabolism, it was thought desirable to try a few experiments to see whether the decrease in regeneration could in any way be correlated with the increase in carbon-dioxide production. The methods employed have already been described p. 469).

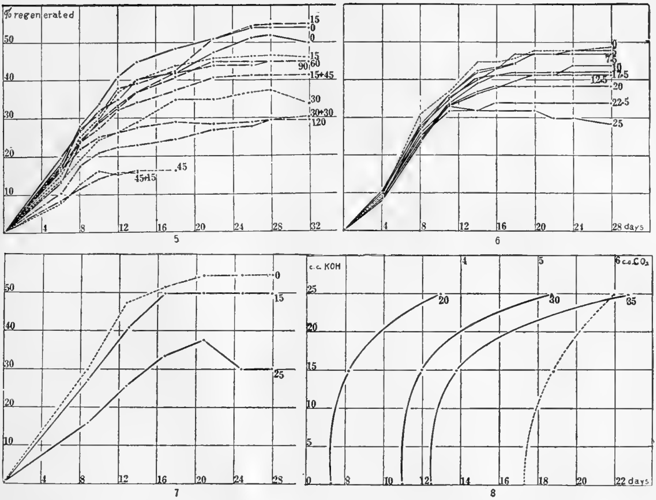


Fig. 5 Graph showing the regeneration of tadpoles in increasing concentrations of Na_2CO_3, $NaHCO_3$, and mixtures of Na_2CO_3 and $NaHCO_3$. *Each line,* average regeneration of four tadpoles; *ordinate,* per cent regenerated; *abscissa,* time in days; *solid lines,* distilled-water controls; *dotted lines,* Na_2CO_3; *dash lines,* $NaHCO_3$; *alternate dot and dash lines,* mixed solutions; *numbers at end of lines,* cc. 0.01 molecular salt solution in 200 cc. of solution used. Where both salts are used, the first number represents the amount of Na_2CO_3. Note that no definite effect of the salt appears until 30 cc. of the Na_2CO_3 or 120 cc. of $NaHCO_3$ has been added, and that the effect of 45 cc. Na_2CO_3 is practically unchanged by the addition of 15 cc. $NaHCO_3$.

Fig. 6 Graph showing the regeneration of tadpoles in increasing concentrations of H_3PO_4. *Dotted line,* control; *ordinate,* per cent regenerated; *abscissa,* time in days; *numbers at ends of curves,* cc. 0.01 mol. H_3PO_4 in 200 cc. of solution. Compare with figures 13, 15, and 17, representing regeneration in corresponding concentrations of HNO_3, HBr, and H_2SO_4.

Fig. 7 Graph showing the regeneration of tadpoles in increasing concentrations of KOH. *Ordinate,* per cent regenerated; *abscissa,* time in days; *numbers at ends of curves,* cc. 0.01N KOH in 400 cc. of solution. The CO_2 production of these tadpoles during the first twenty-four days of regeneration is shown in figures 3 and 8. Tadpoles in higher concentrations died without regeneration. From data of table 3.

Fig. 8 Graph showing the regeneration (solid lines) and CO_2 production (dotted lines) of the tadpoles whose regeneration curves are shown in figure 7. *Ordinate,* concentration as cc. 0.01N KOH in 400 cc. of solution; *abscissa for regeneration,* time in days, *for CO_2 production,* CO_2 produced as cc. 0.01N H_2CO_3 per gram per day; *numbers at ends of regeneration curves,* per cent regenerated. Note the correlation between retardation of regeneration and increase in CO_2 production. From data of table 3.

Table 3 gives the CO_2 production in KOH of six sets of four tadpoles each and the regeneration of the same tadpoles. Those in the higher concentrations (30, 35, and 40 cc. 0.01N KOH per 400 cc.) died without regeneration. Figure 3 (drawn from table 3) represents graphically the increase in CO_2 production with increased concentration of base, and gives a curve almost iden-

TABLE 3

Carbon-dioxide production and regeneration in KOH. *Length of tadpoles 66 to 86 mm.*

0.01 N KOH IN 400 CC. H_2O	0	15	25	30	35	40
Days survived	24.0	24.0	24.0	2.0	1.0	1.0
Weight in grams	6.9	6.9	7.2	7.3	6.8	6.9
Total CO_2 in cc. 0.01 N H_2CO_3	799.3	863.9	1038.4	99.4	48.6	51.3
Average daily CO_2	33.3	36.0	43.25	49.7	48.6	51.3
Average CO_2 per gram per day	4.83	5.22	6.01	6.8	7.14	7.3

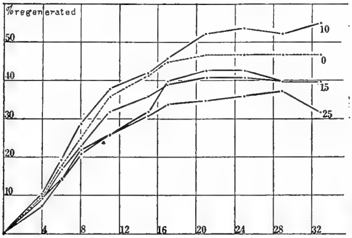
Regeneration

CC. KOH IN 400 CC. H_2O	AVERAGE LENGTH IN MILLIMETERS						
	Removed	Regenerated					
			11th day	16th day	21st day	26th day	36th day
0	12.5	mm.	3.7	5.9	6.4	6.8	7.1
		%	30.0	47.2	51.2	54.4	56.8
15	11.5	mm.	3.1	4.7	5.7	5.7	5.7
		%	27.0	41.0	50.0	50.0	50.0
25	12.1	mm.	1.9	3.1	4.1	4.8	3.9
		%	16.0	25.8	34.0	39.7	32.0

tical with the curve shown in figure 4, which was drawn from the data of Loeb and Wasteneys ('13 b) for the increase in oxygen consumption in increasing concentrations of bases. Figure 7 gives the curves of growth for the three sets of tadpoles which survived to undergo regeneration. It shows clearly the decrease both in the rate of regeneration and the total amount regenerated in increasing concentrations. Figure 8 (drawn from the data of table 3) compares the effect of the various concentrations

of KOH upon the rate of regeneration taken as time necessary to regenerate 20, 30, and 35 per cent of the amount removed. The dotted line represents the average CO_2 per gram per day produced by the same tadpoles. It will be noted that the rate of regeneration curves and the carbon-dioxide curve are not parallel. This, however, would not be expected, as the normal carbon-dioxide production is a straight line—that is, the amount of carbon dioxide produced is practically the same from day to day—whereas the normal regeneration (shown by the control or dotted line in fig. 7) is a curve, increase in length of the regenerating part progressing more slowly as regeneration nears completion. It is of interest to note that the curve of regeneration for the earliest stage shown (20 per cent of the amount removed) corresponds more nearly to the curve of carbon-dioxide production than does the curve for the second stage (30 per cent of the amount removed), and this in turn shows more similarity than does the curve for the third stage (35 per cent of the amount removed). These facts suggest that at the very beginning of the process of regeneration the retardation of regeneration corresponds to the acceleration of oxygen metabolism, but that later the two processes diverge progressively, due to the additional inhibition to regeneration afforded by the presence of the regenerated tissue.

In the next experiment six sets of five tadpoles each were used. The normal CO_2 production in distilled water of each set of tadpoles was first determined for a period of six days, and used as a basis of comparison for the CO_2 production in KOH determined for the five succeeding days. The tadpoles were then operated upon and allowed to undergo regeneration in the same concentrations of KOH. Figure 9 gives the curves of regeneration of the tadpoles which survived to undergo regeneration. Figure 11 compares the CO_2 production expressed as per cent of normal CO_2 production (first dotted line) and as cc. 0.01N H_2CO_3 per gram of tadpole per day (second dotted line) with curves representing the time required by the same tadpoles to regenerate 20, 30, and 35 per cent of the amounts removed in the same concentrations of KOH. While these curves are not comparable,



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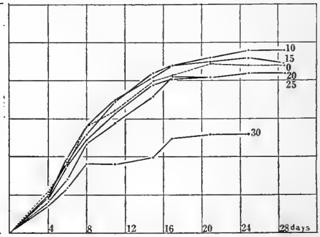

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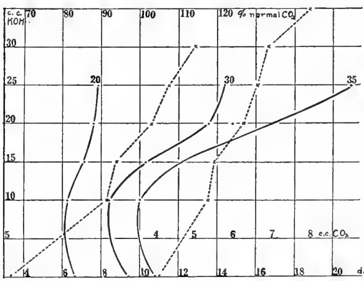

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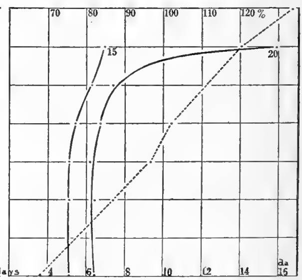

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Fig. 9 Graph showing the regeneration of tadpoles in increasing concentrations of KOH. *Each line,* average regeneration of five tadpoles; *ordinate,* per cent regenerated; *abscissa,* time in days; *numbers at ends of curves,* cc. 0.01N KOH in 400 cc. solution. The CO_2 production of these tadpoles is compared to their rate of regeneration in figure 11. Note the apparently beneficial effect of 10 cc. of KOH. This is probably due to neutralization of CO_2 produced by the tadpoles, as the hydrogen ion concentration of this solution was changed from pH 9 to pH 6.7 within the forty-eight hours elapsing between renewing of the solution.

Fig. 10 Graph showing the regeneration of tadpoles in increasing concentrations of $Ca(OH)_2$. Plotted as figure 9. The CO_2 production of these tadpoles is compared to the retardation of regeneration in figure 12. As in figure 9, the lower concentrations appear to have had beneficial effects.

Fig. 11 Graph showing the per cent of regeneration (solid lines) and per cent of normal CO_2 production (first dotted line) and average CO_2 produced per gram of tadpole per day (second dotted line) in increasing concentrations of KOH of the tadpoles whose curves of regeneration are shown in figure 9. *Ordinate,* concentration as cc. 0.01N KOH in 400 cc. of solution; *abscissa for per cent regenerated,* time in days; *abscissa for per cent CO_2 produced,* per cent of normal CO_2 produced in distilled water; *CO_2 per gram per day,* cc. 0.01N H_2CO_3. Note that whether CO_2 production be expressed as per cent of the normal CO_2 production, previously determined for the same tadpoles in distilled water, or as

the regeneration curves being plotted with abscissa as time and the CO_2 production curves being plotted with per cent of normal CO_2 production and CO_2 produced per gram of tadpole as abscissa, they do serve to show that as CO_2 production is increased, regeneration is decreased progressively. Similar results were obtained in experiments in which the base used was $Ca(OH)_2$. Figure 10 shows the regeneration of these tadpoles. Figure 12 shows the CO_2 production expressed as per cent of normal CO_2 produced in distilled water (dotted line) and the time required to regenerate 15 and 20 per cent of the amount removed.

Child ('13) has shown that for Planaria the rate of regeneration is correlated with rate of oxygen metabolism, and that worms or pieces of worms having high rates of CO_2 production regenerate more rapidly than those having lower rates of CO_2 production. The above experiments upon tadpoles show that a rise in CO_2 production due to the basicity of the medium is correlated with a decrease in regeneration. The apparent discrepancy between these two statements disappears upon considering that the Planaria used by Child were all under optimum conditions for regeneration and growth, but differed only in size and physiological age; whereas of the tadpoles, which were of as nearly as possible the same size and physiological age, only the controls were under optimum conditions for regeneration. Although in case of normal individuals under optimum conditions a rapid oxygen metabolism is often correlated with rapid growth, it is a matter of common knowledge that under certain conditions as a wasting fever or excessive muscular exertion

the amount of CO_2 produced per gram of body weight, there is an increase with increased concentration of KOH and accompanying a retarded regeneration in the higher concentrations.

Fig. 12 Graph showing regeneration (solid lines) and CO_2 production as compared to the normal (dotted line) in increasing concentrations of $Ca(OH)_2$ of tadpoles whose regeneration is shown in figure 10. *Ordinate,* cc. 0.01N $Ca(OH)_2$ in 400 cc. of solution; *abscissa for per cent regenerated,* time in days, *for per cent CO_2 produced,* per cent of normal CO_2 production in distilled water; *numbers at ends of solid lines,* per cent regenerated. As in figure 11, there is an increase in CO_2 production and decrease in regeneration with increased concentration of the base.

there may be a greatly increased oxygen metabolism, which is, however, a destructive metabolism accompanied by cessation of growth or even by regressive changes leading to death. Since in basic media the increased CO_2 production is accompanied by a pathological condition resulting in death, we must regard this high oxygen metabolism also as a destructive metabolism. From this we see the fallacy of using oxygen metabolism as a criterion of what is favorable for general metabolism in judging the suitability of a water for animals. Development, growth, or possibly regeneration must rather be relied upon.

It will be noted that in both of the above experiments the regeneration of the tadpoles in 10 cc. of the base was better than that of the controls. It was, however, observed during the experiment that although the initial hydrogen ion concentration of these solutions was decidedly on the basic side of neutrality (pH about 9), that by the time the solution was changed it was slightly acid (pH about 6.8). For this reason another series of experiments was planned, using the same concentrations, but a larger volume in proportion to the size of the tadpoles, and changing it oftener. Two parallel series were run, each consisting of five small tadpoles in 1 liter of distilled water, 0.00025N NaOH and 0.0005N NaOH (25 and 50 cc. 0.01N per liter). The experiments were carried out in 2-quart mason jars kept tightly sealed, and containing test-tubes of $Ba(OH)_2$ solution to absorb the CO_2 from the air above the water. The results of this experiment are given in table 4. Due to the CO_2 produced by the tadpoles, the distilled water became slightly acid and the NaOH solution less basic; however, it was no longer neutralized. The retardation to regeneration is very marked in 25 cc. per liter, while 50 cc. proved fatal in two days.

These results show that the reason corresponding solutions (5 and 10 cc. 0.01N base in 200 cc.) produced no deleterious effects in the preceding experiments is because the small volumes used were quickly neutralized, and are in accord with the suggestion of Loeb ('04) that the beneficial effect of the addition of a base is due, not to the effect of the hydroxyl ions, but to the neutralization of acids produced by the animal. This further shows that a pH of about 9 is markedly detrimental.

Regeneration in acids

As the previous experiments show, neutrality is more favorable for regeneration in tadpoles than basicity. Is the same true for acidity? A complete series from acids almost certain to have detrimental effects to bases known to be harmful was formed

TABLE 4

Regeneration in NaOH. *Length of tadpoles 37–40 mm.*

cc. 0.01N NaOH PER LITER	LOT	AVERAGE LENGTH IN MILLIMETERS							APPROXIMATE pH	
		Re-moved	Regenerated						Initial	After 24 hours
				4th day	6th day	7th day	12th day	18th day		
0	A	10.5	mm.	1.0	1.9	2.9	3.8	4.5	7	6.5 to 6.6
			%	9.5	18.0	27.7	36.0	43.0		
	B	10.35	mm.	1.0	2.0	2.9	3.8	4.3		
			%	9.7	19.3	28.0	36.5	41.5		
25	A	10.6	mm.	0.7	1.5	2.1	3.1	3.4	8.9 to 9	8 to 8.2
			%	6.6	14.2	19.8	29.0	32.0		
	B	10.3	mm.	0.6	1.2	1.8	2.6	3.0		
			%	5.8	11.7	17.0	25.0	29.2		
50	A	10.1	Died in 3 to 4 days. No regeneration.						Above 10	9 to 9.6[1]
	B	10.3	Died in 3 to 4 days. No regeneration.							

[1] The maximum reduction in pH value occurred the first day of the experiment; the minimum the fourth day, the day in which the tadpoles died.

Total length and readings for tenth and fourteenth days omitted.

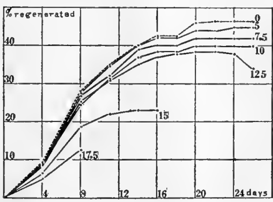
by using 25, 20, 15, 10, and 5 cc. 0.01 mol. H_3PO_4 and 5, 10, 12.5, 15, 17.5, and 20 cc. 0.01N NaOH made up to 200 cc. with distilled water. A control containing 5 cc. of a 0.01 mol. neutral solution of Na_2HPO_4, and KH_2PO_4 was added to the usual distilled-water controls. The solutions were changed daily. It was hoped by this series to locate approximately the optimum hydrogen ion concentration for regeneration.

The results for H_3PO_4 were not very conclusive, as several of the tadpoles died or appeared abnormal. The total amount regenerated was, however, greater in the three controls. The neutral-phosphate control lay about midway between the two distilled-water controls, showing that the small amounts of Na, K, and PO_4 ions employed were neither beneficial nor detrimental. The results of the NaOH series were definite, showing a progressive decrease in both the rate of regeneration and the amount regenerated as the concentration of base increased. While the regeneration of the tadpoles in 5 cc. of NaOH averaged above the controls, the hydrogen ion determinations show that this solution was not only neutralized, but became slightly acid within twenty-four hours, and it has already been shown (table 4) that this same concentration of base is distinctly harmful if the volume is sufficient to prevent it from being neutralized.

The next series of experiments, involving 124 tadpoles, was made in order to compare the effects upon regeneration of the various acids. The experiments were carried on in finger bowls containing 200 cc. of the solution. Four tadpoles were placed in each dish, and the solutions were changed alternate days. The acids used were H_2SO_4, HBr, HNO_3, and H_3PO_4. The tadpoles in the HNO_3 series were slightly smaller than those in H_2SO_4 and H_3PO_4; those in HBr were somewhat larger. The H_2SO_4 series, which is regarded as typical, is given in detail (table 5) in order to show the extent of variation among individuals of a set. About the same extent of individual variation is found in the experiments with bases and other acids.

A comparison of figures 13, 15, and 17 shows that in HNO_3, HBr, and H_2SO_4 the retardation is at first comparatively slight, and increases gradually in concentrations up to 12.5 cc. (0.00065 N). As the concentration rises above this, the retardation increases very rapidly, the order of toxicity of the acids being HNO_3, HBr, H_2SO_4. This might be explained on the ground that in the lower concentrations the acids are so nearly completely ionized as to have about the same effects, but as the concentration is increased the concentration of hydrogen ions, and consequently the toxicity, increases more rapidly in HNO_3 than in

HBr, and in HBr than in H_2SO_4. The data are, however, insufficient to warrant any conclusions. In H_3PO_4 (fig. 6) there was no such marked retardation of regeneration in the concentrations used, although the decrease in the total amount regenerated was equally pronounced. Regeneration proceeded at very



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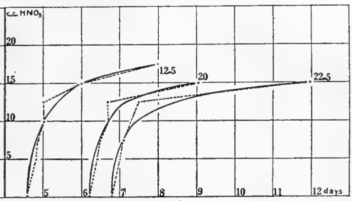

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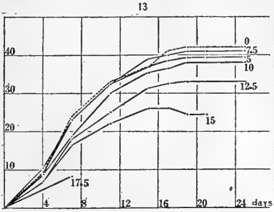

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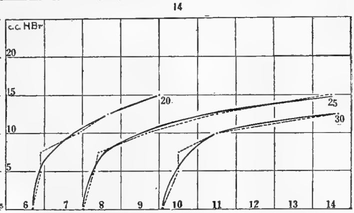

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Fig. 13 Graph showing the regeneration of tadpoles in increasing concentrations of HNO_3. *Each line,* average regeneration of four tadpoles; *dotted line,* control; *ordinate,* per cent regenerated; *abscissa,* time in days; *numbers at ends of curves,* cc. 0.01N HNO_3 in 200 cc. of solution. Compare with figures 6, 15, and 17 for regeneration in H_3PO_4, HBr, and H_2SO_4.

Fig. 14 Graph showing retardation of regeneration in increasing concentrations of HNO_3. *Dotted lines,* experimental data; *solid lines,* theoretical curves; *ordinate,* concentration as cc. 0.01N HNO_3 in 200 cc. of solution; *abscissa,* time in days; *numbers at ends of lines,* per cent regenerated. These curves were plotted from the same data as figure 13. Note that the curves are roughly confocal, showing that the relative retardation for each concentration is the same for the three different stages represented. Compare with figures 8, 11, and 12.

Fig. 15 Graph showing the regeneration of tadpoles in increasing concentrations of HBr. Plotted as figure 13. These tadpoles were slightly larger than those in the HNO_3 experiment.

Fig. 16 Graph showing retardation to regeneration of tadpoles in increasing concentrations of HBr. Plotted as figure 14, from the same data as figure 15. These curves also are roughly confocal, indicating the same relative retardation for each concentration at the three different stages represented.

TABLE 5

Regeneration in H_2SO_4

cc. 0.01 N H_2SO_4 in 200 cc. H_2O		Average length in millimeters												Approximate pH		
		Total	Re-moved	Regenerated										Initial	After 24 hours	After 48 hours
				4th day	8th day	11th day	14th day	16th day	18th day	20th day	22d day	24th day	26th day			
0		41	11.0	1.0	3.0	4.0	5.0	5.0	4.8	4.8	4.8	5.0	5.0	7.0	6.8	6.8
		43	10.7	1.0	3.5	4.3	4.7	4.9	5.1	5.3	5.0	5.0	5.0			
		40	10.0	1.0	3.4	4.0	4.7	4.9	5.0	5.3	5.5	5.2	5.5			
		40	10.8	1.0	3.3	3.6	4.5	4.6	4.6	4.8	4.8	5.0	4.9			
	Av.	41	10.65	1.0	3.3	4.0	4.8	4.8	4.9	5.0	5.0	5.1	5.1			
	%			9.4	31.0	37.5	45.0	45.0	46.0	48.0	47.0	48.0	48.0			
5.0		46	10.0	1.2	3.6	4.0	4.5	4.5	4.5	4.8	4.8	4.7	5.0	5.5	6.4	6.5
		43	10.2	0.8	3.0	3.8	4.4	4.3	4.4	4.6	5.0	5.0	5.1			
		40	11.0	1.0	2.9	3.5	4.5	5.0	4.8	5.0	5.2	5.2	4.7			
		38	11.0	0.9	3.0	3.9	5.1	5.1	5.1	5.3	5.3	5.3	5.3			
	Av.	41	10.7	1.0	3.1	3.8	4.6	4.7	4.7	4.9	5.0	5.0	5.0			
	%			9.3	29.0	35.5	43.0	44.0	44.0	46.0	47.0	47.0	47.0			
7.5		36	10.0	1.1	3.0	3.8	4.6	5.0	5.0	5.1	5.0	5.0	5.0	4.5	6.0	6.3
		33	11.0	1.1	2.9	3.8	4.1	4.7	4.3	4.9	4.9	4.9	5.0			
		36	9.4	1.0	2.1	3.0	3.5	3.5	3.7	4.0	4.0	4.0	4.0			
		38	11.0	0.9	2.2	3.2	3.0	3.0	3.4	3.6	3.6	3.6	3.5			
	Av.	36	10.12	1.0	2.55	3.4	3.8	4.1	4.1	4.4	4.4	4.4	4.4		–	–
	%			9.9	25.3	34.0	37.5	40.4	40.5	43.5	43.5	43.5	43.5			
10.0		43	10.1	0.8	3.0	3.4	4.0	4.1	4.3	4.5	4.5	4.5	4.5	3.9	5.5	6.1
		39	10.0	0.8	2.5	3.8	4.1	4.5	4.1	4.3	4.4	4.4	4.4			
		37	10.2	0.9	2.3	3.2	3.3	3.3	3.8	4.1	4.1	4.1	4.1			
		38	11.0	0.9	2.2	3.2	4.2	4.3	4.0	4.5	4.4	4.4	4.4			
	Av.	39	10.3	0.9	2.5	3.4	3.9	4.0	4.1	4.3	4.3	4.3	4.3			
	%			8.7	24.0	33.0	37.8	39.0	40.0	42.0	42.0	42.0	42.0			

12.5		42	10.0	0.6	2.2	3.0	4.0	4.1	4.0	4.5	4.5	4.5	4.0	3.4	4.9	5.5
		43	10.0	1.2	2.6	3.0	3.8	4.0	4.0	4.1	4.2	4.0	4.5			
		37	10.5	1.0	2.8	3.4	3.5	3.5	3.5	3.7	4.0	4.0	4.0			
		36	10.3	0.8	2.0	2.4	4.0	4.0	4.0	4.1	4.5	4.5	4.5			
	Av.	39	10.2	0.9	2.4	2.9	3.8	3.9	3.9	4.1	4.3	4.3	4.3			
	%			8.8	23.5	28.0	37.0	38.0	38.0	40.0	42.0	42.0	42.0			
15.0		40	10.0	1.0	2.8	3.1	4.0	4.1	3.7	3.7	3.7	3.7	3.7	3.3	4.4	5.0
		41	10.9	0.8	1.7	3.0	3.7	3.8	3.5	3.6	3.4	Dead				
		41	11.0	0.8	2.3	3.0	3.4	3.6	3.6	3.6	3.6	3.6	Dead			
		35	10.2	0.9	2.0	2.2	2.5	2.6	3.0	3.0	3.0	3.0	Dead			
	Av.	39	10.5	0.9	2.2	2.8	3.4	3.5	3.5	3.5	3.4	3.4				
	%			8.5	21.0	27.0	32.0	33.0	33.0	33.0	32.0	32.0				
17.5		39	11.0	1.0	1.6	Dead								3.2		
		43	10.4	0.5	1.2	1.3	1.3	1.3	Dead							
		36	10.6	1.0	1.7	1.7 (T)	2.5	3.0	3.0	3.1	Dead					
		36	10.1	0.7	1.1	1.1	Dead									
	Av.	38	10.5	0.8	1.4	1.4	1.3	1.3								
	%			7.6	13.3	13.3	12.5	12.5								
20.0		40	10.4	0.7	Dead											
		38	11.0	0.3	1.3 (T)	1.3	1.7	2.0	1.8	2.1	2.1	2.3	2.3	3.0		
		36	10.0	0.5	1.5	Dead										
		35	10.1	1.0	Dead											
	Av.	37	10.4	0.6	1.4											
	%			5.8	13.4											

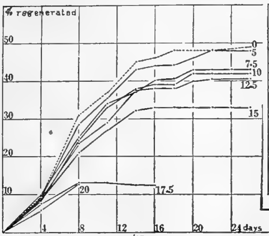
(T) indicates tadpole was transferred to neutral distilled water.

nearly the normal rate up to a certain point, and was then discontinued abruptly. Other experiments using H_3PO_4 gave similar characteristic results.

During the experiment a few tadpoles were transferred to neutral distilled water to see whether normal regeneration could take place after the initial stunting. Their subsequent regeneration is, of course, not added in with that of the other tadpoles of the group in determining the average per cent of regeneration. Tadpoles transferred during the first days of the experiment underwent more regeneration than those left in the acids, but their regeneration was far below normal. At the close of the experiment all survivors were transferred to distilled water, but none of them underwent further regeneration, showing that the effect of acids is not merely a retardation, but a permanent inhibition to regeneration.

The curves in figure 19 represent the time required to regenerate 22.5 per cent of the amount removed in HNO_3, and 25 per cent of the amount removed in HBr, H_2SO_4, and H_3PO_4. The dotted line represents the actual experimental data, the solid line the theoretical curve. Except in the case of H_3PO_4, these lines coincide very closely.

Figures 14, 16, and 18 are curves giving the time necessary to complete the regeneration of various per cents of the amounts removed in H_2SO_4, HBr, and HNO_3, respectively. A comparison of these with the curves for the regeneration in bases of various per cents of the amounts removed shows that although in one case (fig. 11) the regeneration in higher concentrations is irregular, in general the curves are similar. In any given series all of the curves of regeneration—which represent different stages in the regeneration of the same animals—are roughly confocal. This means that while the rate of regeneration at different stages is different, regeneration progressing more slowly as it nears completion, the per cent of retardation due to acids or bases as compared to normal regeneration is the same for all stages, or that for each concentration the relative velocities for the different stages are practically the same. In other words, if the times required by tadpoles in any given concentration of an acid or



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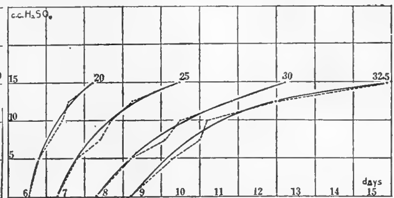

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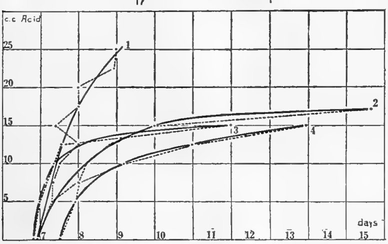

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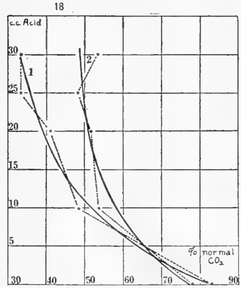

20

Fig. 17 Graph showing the regeneration of tadpoles in increasing concentrations of H_2SO_4. *Each line,* average regeneration of four tadpoles; *dotted line,* controls; *ordinate,* per cent regenerated; *abscissa,* time in days; *numbers at ends of curves,* cc. 0.0LNH_2SO_4 in 200 cc. of solution. From data of table 5.

Fig. 18 Graph showing retardation of regeneration of tadpoles in increasing concentrations of H_2SO_4. *Dotted lines,* experimental data; *solid lines,* theoretical curves; *ordinate,* concentration as cc. 0.01N H_2SO_4 in 200 cc. of solution; *abscissa,* time in days; *numbers on curves,* per cent regenerated. From data of table 5. These curves are comparable to those shown in figures 8, 11, 12, 14, and 16.

Fig. 19 Graph comparing regeneration in increasing concentrations of acids. *Curve 1,* H_3PO_4, 25 per cent regenerated; *curve 2,* H_2SO_4, 25 per cent regenerated; *curve 3,* HNO_3, 22.5 per cent regenerated; *curve 4,* HBr, 25 per cent regenerated. Plotted as figure 18 from the same data as figures 6, 13, 15, and 17. The action of HNO_2, HBr, and H_2SO_4 is similar throughout. The acid action of H_3PO_4 seemed to be obscured by its toxicity in higher concentrations.

Fig. 20 Graph showing the CO_2 production of tadpoles in increasing concentrations of HBr (curve 1) and HCl (curve 2). *Ordinate,* concentration as cc. 0.01N acid in 400 cc. of solution; *abscissa,* per cent of normal CO_2 produced in distilled water. The regeneration of these tadpoles was progressively inhibited as the concentration of acid increased.

base to regenerate 20, 25, and 30 per cent, etc., of the amounts removed be divided by the times required by the controls to regenerate the corresponding amounts, the results will be approximately the same. The inhibitory action of the base or acid is, in general, no greater at one stage than at another. This is similar to the findings of Krogh ('14 a) in his work on the development of frog's eggs at various temperatures.

Carbon-dioxide production and regeneration in acids

The next series of experiments was undertaken to see whether any correlation could be found between regeneration and carbon-dioxide production in acids. Ten sets of four tadpoles each of as nearly the same size as possible were used. The normal carbon-dioxide production in distilled water for a period of three days was determined after a two-day fast to allow the alimentary tract to become empty. The first and tenth sets were then kept in distilled water as controls, and the other eight put into 10, 20, 25, and 30 cc. of HCl and HBr made up to 400 cc. with distilled water. The carbon-dioxide production, whether expressed as cc. 0.01N H_2CO_3 per gram per day or as per cent of normal, decreases progressively with the increase in concentration of acids except in case of the tadpoles in 30 cc. of HCl (fig. 20) which is probably not typical. The survivors were allowed to regenerate in the same concentrations of acids, and again showed a progressive decrease in regeneration from the lower to the higher concentrations. Those in 25 cc. of acid showed very little regeneration, and survived only eight days.

The effect of the tadpoles upon the medium should be mentioned in this connection. It will be noted that although the titratable acidity of the acid solutions (determined as $Ba(OH)_2$ neutralized) becomes greater, due to the acids produced by the tadpoles, the hydrogen ion concentration of the solution decreases. It was furthermore noted, throughout all of the experiments, that tadpoles in strongly acid solutions decrease greatly in size and much more rapidly than those in strongly basic solutions, notwithstanding the fact that the carbon-dioxide production of the

latter is much greater. Osterhout ('14) has shown that the effect of acids upon a living membrane is to cause an initial decrease in permeability followed at once by a rapid increase which continues to the point of death. The decrease in size of tadpoles in the strongly acid solutions may be attributed to the loss of substances from the tissues due to the increased permeability of the membranes. If these substances are, as is probably the case, largely in the form of phosphates and amphoteric substances such as amino acids, their effect upon the medium would be to decrease the hydrogen ion concentration, as shown by the indicators, by forming with the acids of the solution, new compounds which ionize differently. This, however, would not reduce the titratable acidity as shown by the power of the solution to neutralize a strong base such as $Ba(OH)_2$.

That the effects of acids are due to the free H ions, and not to the titratable acidity, was suggested by the observation, made throughout the experiments, that tadpoles which died usually succumbed within a few hours after the solution was changed, and that many tadpoles which showed symptoms of severe disturbances when put into the fresh solution, gradually became more normal during the twenty-four or forty-eight hours before it was changed again. This was not due to acclimation, for with the next changing of the solution they again showed the same pathological symptoms and frequently succumbed. But one experiment was performed in this connection: Ten tadpoles of about 55-mm. length were placed in 1 liter of 0.00075N HCl (75 cc. 0.01N HCl per liter). The pH of this solution was about 3.4. After thirty-six hours six of the tadpoles were dead and the other four were in a dying condition. These tadpoles were removed and two fresh tadpoles were placed in the same solution which now had a pH of about 5.3. At the same time two similar tadpoles were placed in a freshly prepared HCl solution. The next morning the two tadpoles in the fresh solution were both dead. Those in the solution in which others had died were still alive at the end of two weeks, at which time the experiment was discontinued. Titration of a sample of the solution made on the third day showed no decrease in its ability to neutralize

$Ba(OH)_2$, hence the addition of substances to the water which will decrease the number of free H ions is beneficial to the tadpoles regardless of titratable acidity.

Table 6 gives the results of an experiment in which CO_2 determinations were made daily throughout the period of regeneration.

TABLE 6

Regeneration in HCl

cc. 0.01 N HCl in 200 cc. H_2O	LENGTH IN MILLIMETERS											
	Total	Re-moved	Regenerated									
				14th day	21st day	28th day	33d day	38th day	42d day	46th day	51st day	53d day
0	75	13.2	mm.	1.0	1.7	2.6	3.0	3.5	4.0	4.5	4.5	4.5
			%	7.6	12.9	19.7	23.0	26.3	30.0	34.0	34.0	34.0
5	71	12.5	mm.	0.6	1.3	1.9	2.3	2.9	3.2	3.2	3.2	Dead
			%	4.7	10.4	15.2	18.4	23.0	25.6	25.6	25.6	
10	72	12.0	mm.	0.5	0.8	1.8	2.3	2.6	2.9	3.0	3.0	Dead
			%	4.2	6.6	15.0	19.0	21.8	24.0	25.0	25.0	
15	68	12.1	mm.	0.4	0.5	0.8	0.8	1.0	Dead			
			%	3.3	4.1	6.6	6.6	8.3				

CO_2 production during the period of regeneration

cc. 0.01 N HCl IN 200 cc. H_2O	pH	AVERAGE NORMAL DAILY CO_2 PRODUCTION	SURVIVAL DAYS	CO_2 PRODUCED IN ACID AS 0.01 N H_2CO_3		PER CENT NORMAL CO_2
				Total	Average per day	
0	7.0	6.9	53	333.9	6.3	91.3
5	5.4	5.69	53	254.1	4.8	84.3
10	3.8	6.75	51	185.8	3.64	53.9
15	3.2	6.17	45	105.35	2.34	37.9

Four rather large tadpoles were used, care being taken to have them as similar as possible. The experiment was begun at an almost constant temperature of 13°C., but after nineteen days was placed at room temperature in order to hasten regeneration. The effect of the acid in decreasing both the CO_2 production and the regeneration is pronounced.

Relation of size to carbon-dioxide production and regeneration

It has already been mentioned in connection with earlier experiments (p. 474) that there is a difference between large and small tadpoles in the relation of retardation of regeneration and length of life in various concentrations of bases. Dreyer and Walker ('14) show that in warm-blooded animals of the same species, but of different weights, dosage of drugs must be calculated in relation to body surface rather than weight. They explain this on the ground that "The concentration in the plasma of any given substance administered is dependent on the volume of the circulating blood, which is itself proportional to the body surface in any given species of animal." It is also known that the 'basal heat production' of a warm-blooded animal, which may be defined as the heat produced by the animal when kept at the same temperature as its normal body temperature, at rest and starving, is proportional to body surface for any given species.

A few experiments were undertaken to see whether any relationship could be traced between the body area or body weight of tadpoles and the effects of acids and bases upon regeneration and carbon-dioxide production, which may be regarded as most nearly corresponding to the basal heat production of mammals.

For the first experiment two tadpoles were used which had been collected at the same time and differed only in size, great care being taken that they should be similar in shape, relative length of tail, etc. After one week of starvation in distilled water they were weighed, and the CO_2 production of each was determined for three successive days as follows:

WEIGHT OF TADPOLE	CO_2 PRODUCED AS CC. 0.01 N H_2CO_3				
	1st day	2d day	3d day	Total	Per gram
grams					
1.6	2.35	1.7	2.3	6.35	3.97
0.86	1.05	1.05	1.3	3.4	3.99

While the CO_2 production per gram of body weight was not the same for the two specimens on any one day, the averages of the three days are practically identical.

For the next experiment a new method of calculating the areas was devised. The average of the length, width, and depth of the body was found, and with this as the diameter the area of the body was calculated as a sphere. The area of the tail was computed by regarding it as a rectangle from the base to the level at which it begins to taper rapidly to a point, and as a triangle from this level to the tip. These areas were doubled to give the two sides of the tail, and added to the area of the body, already found, to give the total area of the tadpole which is expressed in square millimeters. The area computed by this method is probably considerably less than the actual area of the tadpole, but it was hoped that by selecting animals similar in shape, the ratios of computed area to actual area would be about the same.

Three sets of three tadpoles each were measured, weighed, and the CO_2 production in distilled water determined for six successive days with the following results:

WEIGHT OF TADPOLES	Area	CO_2 PRODUCED IN SIX DAYS AS CC. 0.01 N H_2CO_3					
		Total	Average per day	Average per gram per day	Per cent area	Per cent weight	Per cent CO_2
grams	*sq. mm.*						
12.0	5563	328.4	54.73	4.56	100.0	100.00	100.0
7.7	4041	215.6	39.96	4.66	74.1	64.17	65.3
3.5	2501	105.1	17.51	5.0	44.6	29.17	32.0

The CO_2 per day per gram of tadpole, 4.56 cc. for the larger size, 4.66 cc. for the second size, and 5 cc. for the smaller size, shows a slight increase in CO_2 production per unit of weight with a decrease in size. In order to compare the relative area, weight, and CO_2 production of the three tadpoles, all were expressed in terms of per cent of the larger size, taken as 100 per cent (last three columns). The weight and CO_2 production vary by differences of $+1.3$ per cent and $+2.83$ per cent in the second and third sizes, respectively, while the area and CO_2 production vary by differences of -8.8 per cent and -12.5 per cent, suggesting that the normal CO_2 production of tadpoles in distilled water is much more nearly correlated with body weight than with area, as is the case for warm-blooded vertebrates.

The next series was carried out in the same manner. Carbon-dioxide production was determined over a period of eight days. It was noticed during this experiment that the large tadpoles frequently exhausted the oxygen from the air in the experimental jars. This was evident because when the jars were opened, although the temperature had remained constant, the lids came off with a 'pop,' showing that a partial vacuum had been formed. The tadpoles also had a tendency to float at the surface as they had been observed to do in low oxygen water. For this reason it was rather to be expected that the CO_2 production of the larger size would be proportionately low, and this was found to be the case as the following table shows:

SIZE	WEIGHT	AREA	CO_2 PRODUCED IN DISTILLED WATER AS CC. 0.01 N H_2CO_3 (EIGHT DAYS)					
			Total	Per day	Per gram day	Per cent area	Per cent weight	Per cent CO_2
	grams	*sq. mm.*						
Large.....	11.2	5261	324.3	40.5	3.61	100.0	100.0	100.0
Medium..	5.1	3119	169.8	21.2	4.16	59.2	45.5	52.3
Small.....	2.0	1607	72.1	9.0	4.5	30.5	17.9	22.2

When, now, the large tadpoles, whose CO_2 production was limited by insufficiency of oxygen, are taken as standard or 100 per cent in comparing the area, weight, and CO_2 production of the three sets, the CO_2 production of the second and third sets appear abnormally high. Despite this fact, however, the CO_2 production still corresponds more nearly to weight than to area, especially in the third size.

At the close of the eight days in distilled water the CO_2 production of the same tadpoles was determined for a period of six days in 0.0005 N HCl (20 cc. 0.01 N in 400 cc.). The CO_2 production decreased in all three sets. Although the absolute decrease was greater in the larger tadpoles the relative decrease was greater in the smaller ones, the per cent of decrease as compared to normal being 38 per cent, 48 per cent, and 50 per cent in the large, medium, and small sizes, respectively.

SIZE	NUMBER OF DAYS	CO_2 IN 0.0005 N HCl AS 0.01 N H_2CO_3			DECREASE FROM NORMAL CO_2			
		Total	Per day	Per gram day	Per day	Per cent weight	Per cent area	Per cent decrease
Large.....	6	151.1	25.2	2.25	15.3	100.0	100.0	100.0
Medium..	4	44.0	11.0	2.16	10.2	45.5	59.3	66.6
Small.....	3	13.5	4.5	2.25	4.5	17.85	30.5	30.0

If decrease in CO_2 production, weight, and area be compared, using the large size as 100 per cent, the decrease in CO_2 production appears to be fairly well correlated with area. This evidence is, however, offset by the fact that the daily CO_2 production per gram of tadpole is practically the same for all three sets as though the lowered oxygen metabolism produced by the acid had balanced the shortage of oxygen in the experimental jars of the larger tadpoles mentioned above.

Another series was attempted in which the error due to the large tadpoles exhausting the air in their jars was avoided by putting the same weight rather than the same number of tadpoles in each jar; thus a jar might contain one large tadpole, three medium-sized tadpoles, or six small ones. Four sets of each size were weighed and measured. The CO_2 production in dis- illed water, which was then determined for each set for six successive days, varied from 5.65 to 6.45 cc. 0.01 N H_2CO_3 per gram per day. This variation bore no relation to size, as the extremes were both found in the largest size. The CO_2 production of the smallest size varied from 5.7 to 6.2 cc. per gram per day. The average CO_2 production per gram per day was for the largest size 6.13 cc.; for the second size 6.04 cc., and for the small size 5.96 cc. In view of the wide variation in CO_2 production between different sets of tadpoles of the same size, the differences in these numbers are probably not significant.

After the determination of normal CO_2 production in distilled water, one set of each size was continued in distilled water as a control, and one set of each size was put into 0.0005 N KOH, $Ca(OH)_2$, and H_2SO_4, and the CO_2 production determined for four successive days. Due to the early death of some of the

tadpoles and the abnormal behavior of others during the experiment, no attempt was made to correlate the increase of CO_2 production in bases or the decrease in acids with either weight or area. The data show, on the whole, that the per cent increase of CO_2 production in bases and the per cent decrease in acids both increase as the size of the tadpoles decreases.

Child ('18) shows that in Gonionemus the effects of abnormal hydrogen ion concentrations upon metabolism decrease as age increases. In the majority of the author's experiments, however, there was no indication that the tadpoles differed in age or nearness to metamorphosis, and the few tadpoles which did show rudimentary hind legs did not differ appreciably nor always in the same direction from the physiologically younger tadpoles of the same size. Experience in rearing tadpoles shows great variation in size in animals of the same age. In view of these facts, it would seem that until parallel experiments can be performed upon animals of the same size, but differing in age, and of the same age, but differing in size, it must be regarded as an open question whether the relatively greater effect of abnormal hydrogen ion concentrations upon CO_2 production in smaller tadpoles may not be due simply to the relatively larger area exposed to the unfavorable medium.

The surviving tadpoles of the preceding experiment were operated upon and allowed to undergo regeneration in the same solutions as the CO_2 determinations had been made for. The concentrations used had no effect upon regeneration in the largest size. The medium-sized tadpoles were affected only in the case of H_2SO_4 in which regeneration was noticeably retarded, while in the few of the smaller tadpoles which survived to regenerate the regeneration was markedly depressed.

A final series on the effect of size on regeneration was run in 30, 40, 50, and 60 cc. 0.01 N HCl made up to 1 liter in 10-inch crystallizing dishes, each containing ten tadpoles of three distinct sizes. Two controls were run. All of the tadpoles in the highest concentration died without regeneration. Throughout the experiment the smaller tadpoles showed a higher per cent of regeneration than the larger ones until a concentration was reached in

which most of them died. In such a concentration the few which survived underwent very little regeneration. In a concentration in which most of the tadpoles lived, however, if the per cent regenerated by each tadpole be divided by the per cent regenerated by its respective control, the result is approximately the same for all sizes. This would indicate that the effect of an acid upon regeneration is relatively independent of the size of the tadpole; that the effect is probably largely local upon the regenerating tissue until a concentration is reached which seriously affects the tadpole as a whole, after which regeneration is retarded by the acid both directly and indirectly because of the impaired health of the parent animal, upon which the new growing tissue is dependent for nutriment. This point is reached in the smaller individuals before it is in the larger ones.

These experiments, while few in number, would suggest the use of abnormal conditions, such as those produced by chemicals, as a promising method of attack for investigations upon the relation between new tissues and the organism as a whole.

Regeneration in low oxygen

The method of obtaining a gradient of oxygen has already been described (p. 466). For the first series of experiments three tadpoles of different sizes were placed in each of the seven experimental jars. During this experiment the water was strongly basic to phenolphthalein, though acid to thymolphthalein. Of the larger tadpoles, 70 to 77 mm., the one in the first jar (oxygen-free water) died without regeneration, and no. 3—in 1.6 cc. O_2 per liter—showed better regeneration than nos. 5 and 6—in 3.03 and 3.8 cc. O_2 per liter, respectively. This is doubtless accounted for by the fact that nos. 2 and 3 remained most of the time at the surface of the water, while nos. 4, 5, 6, and 7 remained at the bottom, coming to the top only occasionally. When the fact that the water in which nos. 2 and 3 actually remained contained considerably more oxygen than was shown by the titrations is taken into consideration, these data show clearly a relation between regeneration and the oxygen content of the water. The same thing is true of the medium-sized tadpoles.

Of these nos. 1 and 3 remained at the top and underwent regeneration. No. 2, which did not show this response, died without regeneration. Nos. 4, 6, and 7, which remained at the bottom, underwent progressively better regeneration. The results for the small tadpoles, 34 to 37 mm., are quite typical of what occurred each time an attempt was made to use small tadpoles in oxygen experiments. Nos. 1, 2, and 3 remained at the top of the water. Nos. 2 and 3 lived twelve days and underwent slight regeneration. Nos. 4, 5, 6, and 7 stayed at the bottom, and of these only no. 7 survived to undergo any regeneration, but its regeneration was apparently normal.

Here again, as was shown for the acids and bases, we find the susceptibility of the tadpole as a whole as compared to the susceptibility of the regenerating part higher in smaller than in larger tadpoles.

All subsequent experiments were carried on in water kept basic to rosalic acid, and from acid to slightly basic to phenolphthalein. The temperature was also corrected by the introduction of the air coil which warmed the water to room temperature before it entered the experimental jars. All of the tadpoles chosen were large and showed rudimentary hind legs, as it was found that tadpoles at this stage remain at the bottom of the jars better than those in earlier stages. The oldest tadpoles used had a total length of 75 to 85 mm., and the hind legs were two-jointed and digited, and stood out from the body so as to be visible from the dorsal aspect. The results of this series (table 7, fig. 21) again show a close correspondence between the amount of oxygen and the rate of regeneration.

Figure 23 is a curve drawn from these same data representing the length of time necessary to regenerate 20 per cent of the amount removed in increasing concentrations of oxygen. A comparison of this curve with the curves of Krogh ('14 a, b) for increase in the rate of development at increased temperatures and the toxicity curves of Powers ('18 b) for the decrease in length of life with increase in concentration of toxic substances shows that they are only in part similar. If the author's curves for retardation of regeneration in bases and acids and for increased

carbon-dioxide production and oxygen consumption in bases, and Powers' curve for oxygen consumption in increasing amounts of anaesthetics had been drawn with the maximum concentration used (that is, a concentration just sufficient to prevent regeneration or to cause the death of the organism) taken as the zero of the ordinate instead of with the control, or absence of the harmful factor, as zero all of the curves mentioned would be somewhat similar. In all the theoretical zero falls far below the actual zero.

The final experiment of the oxygen series owes its value to an accident. The tadpoles of this series were 71 to 76 mm. long and

TABLE 7

Regeneration in low oxygen

CC. O_2 PER LITER			LENGTH AS MILLIMETER		AMOUNT REGENERATED							
Maximum	Minimum	Average	Total	Removed	12th day		17th day		25th day		31st day	
					mm.	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*
Trace	0	0+	83	22.5	4.0	17.5	4.5	20.0	4.8	21.3	5.3	23.5
1.0	0.2	0.6	79	20.0	3.0	19.0	4.8	24.0	5.5	24.8	5.5	27.5
1.9	0.45	1.17	81	22.2	4.3	19.4	5.5	24.8	6.7	30.0	6.9	31.0
3.3	1.0	1.8	85	23.0	5.0	21.7	5.9	25.6	6.9	30.0	7.2	31.3
3.6	1.8	2.7	80	21.6	5.4	25.0	6.1	28.0	6.3	29.0	6.3	29.0
4.68	2.3	3.28	82	20.7	5.6	27.0	6.1	29.0	7.8	36.7	7.6	36.7
5.7	3.1	4.1	82	22.0	6.2	28.0	7.6	34.5	8.5	38.5	8.4	38.0

had rudimentary hind legs. No. 3 died without regeneration. Of the other five, nos. 1 and 2, in 0.2 and 1.5 cc. O_2 per liter, respectively, showed marked retardation of regeneration when measured on the twenty-first day (fig. 22). During the twenty-second night the flow of water through the jars stopped, due to clogging of the boiling apparatus by sediment from the water, and before this could be remedied the water in the jars had become well aerated. Since the tadpoles had been for two days in aerated water, the original purpose of the experiment had to be abandoned. An aerating jar with a rapid stream of air passing through it was placed just after the mixing bottle so that the water entered the first experiment jar aerated. Measurements made

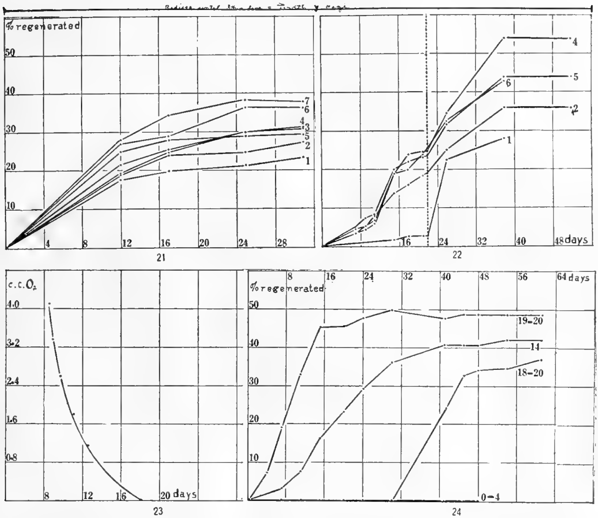

Fig. 21 Graph showing the regeneration of tadpoles in increasing concentrations of oxygen. Each line represents the regeneration of one individual tadpole. Numbers at the ends of lines correspond to position in the series of increasing oxygen content (table 7). *Ordinate,* five regenerated; *abscissa,* time in days. From data of table 7.

Fig. 22 Graph showing the regeneration of tadpoles in increasing concentrations of oxygen and the result of aerating the water before the completion of regeneration. Plotted as figure 21. Dotted line indicates the time at which the water became aerated.

Fig. 23 Graph showing the time required to regenerate 20 per cent of the amount removed in increasing concentrations of oxygen. *Ordinate,* cc. oxygen per liter; *abscissa,* time in days. From data of table 7. Since regeneration increases with concentration of oxygen and decreases with increase in concentration of bases and acids, this curve is the converse of the retardation of regeneration curves shown in figures 8, 11, 12, 14, 16, and 18, although plotted the same. It conforms quite closely to an equilateral hyperbola with its zero a minus amount of $oxygen_2$

Fig. 24 Graph showing the regeneration of tadpoles in increasing temperatures. *Each line,* average of four tadpoles; *ordinate,* per cent regenerated; *abscissa,* time in days; *numbers on curves,* temperature centigrade. From data of table 8. The tadpoles transferred from the lowest temperature (0° to 4°) on the thirtieth day undergo a regeneration parallel to those put at room temperature immediately after operation. The regeneration is, however, not so complete.

on the twenty-sixth day of the experiment showed that the tadpoles in the first jar, which had regenerated only 2.8 per cent during the twenty-two days preceding the aeration of the water, had regenerated 19.8 per cent in the four days following it. The tadpoles previously in higher amounts of oxygen, of course, showed less difference. This experiment is of value as demonstrating that the increase in the higher amounts of oxygen is due to the oxygen, and not to any slight rise in temperature in the successive jars, or to a decrease in the alkalinity of the water as it takes up carbon dioxide produced by the successive tadpoles.

Regeneration in low temperatures

It has long been known that low temperatures, if not so low as to produce detrimental effects, may retard or completely check the development of certain animals for a considerable period of time, the animal still retaining power to complete normal development. The following experiment was planned to test whether this might be true for regeneration. Three sets of four tadpoles each were selected. The first set was kept at room temperature—19° to 21°C.; the second was kept in a water-bath at 14°, and the third was kept in a refrigeration tank at 0° to 4°. The first set underwent regeneration rapidly, the rate of the second set was considerably retarded, the third set underwent no regeneration whatever (table 8, fig. 24). When the first set had completed regeneration, two from the third set were transferred to room temperature. The day in which they were transferred and several succeeding days were cool, so the temperature of the water stood at 18° to 19°. For this reason, probably, the regeneration of these tadpoles was slightly slower than that of the controls, although the growth curves are similar. The two tadpoles left at the lower temperature died without regeneration at the end of forty-seven days.

GENERAL DISCUSSION

In comparing the effects upon metabolism of abnormal hydrogen ion concentrations, low oxygen, and low temperature, it is of interest to note that alike they cause decrease in both the rate of regeneration and the total amount regenerated. Were the effects of these agents due wholly to their action in retarding the metabolism and consequent division and growth of the cells of the regenerating parts, the result would not be a decrease in the total amount ultimately regenerated, but rather an increase

TABLE 8

Effect of temperature on regeneration

t.C.	AVERAGE LENGTH IN MILLIMETERS														
	Total	Removed	Regenerated												
				4th day	7th day	11th day	15th day	20th day	24th day	30th day	41st day	45th day	48th day	54th day	59th day
19 to 20	58	15.15	mm.	1.1	2.95	5.0	6.9	7.0	7.0	7.3	7.3	7.4	7.4	7.4	7.4
			%	7.3	19.40	33.0	45.5	46.0	46.0	48.0	48.0	49.0	49.0	49.0	49.0
14	61	15.0	mm.		0.50	1.2	2.5	3.5	4.5	5.5	6.1	6.2	6.2	6.3	6.3
			%		3.30	8.0	16.8	23.0	30.0	36.0	41.0	41.0	41.0	42.0	42.0
0 to 4	60	15.4												Dead	
	59	15.25	mm.							T	3.7	5.0	5.2	6.1	6.4
			%								24.3	33.0	34.0	40.0	42.0

'T" = transferred to temperature 18° to 21°C.

in the length of time necessary to complete regeneration. This, however, is not the case, for in acids and bases the tadpoles in the higher and more detrimental concentrations cease regenerating at a time as early or earlier than the controls. In low oxygen regeneration ceases at practically the same time in all concentrations, while in low temperatures, although the period of growth is longer at the lower than at the higher temperatures, the difference is not sufficient to compensate the difference in rate of regeneration, so that the ultimate amount regenerated is still less at the lower than at the higher temperatures.

It was further noticed throughout these experiments that the appearance of pigment in the regenerated part, which may be supposed to show a certain stage of differentiation, occurred as early or earlier in the tadpoles in high concentrations of acids or bases as in the controls; at about the same time in the low oxygen water, and somewhat later in the low temperatures. From this it would seem that growth and differentiation (if appearance of pigment be accepted as a criterion of differentiation) in the regenerating parts are differently affected by the environmental agents studied, so that a hydrogen ion concentration, an oxygen content of the water, or a temperature of the surrounding medium which retards one process may have little or no effect upon the other. If, then, as is frequently supposed, the presence of differentiated tissue be the inhibitory factor which causes the cessation of growth of the new part, this difference in effect upon growth and differentiation by the environmental factors considered would account for the differences in the ultimate amount regenerated in the various concentrations of hydrogen ions and oxygen or at the various temperatures.

SUMMARY

A study has been made of the effects of various conditions of hydrogen ion concentration, deficiency of oxygen and low temperature upon regeneration and oxygen metabolism in tadpoles of Rana clamata.

1. These studies show that the optimum hydrogen ion concentration for regeneration is neutrality, or near neutrality, probably between pH 6.7 and pH 7.6. As the hydrogen ion concentration varies from the optimum in the direction of either acidity or basicity, both the rate of regeneration and the total amount regenerated decrease, at first gradually and then very rapidly (figs. 1, 2, 5, 6, 9, 10, 13, 15, and 17).

2. The relative effect upon regeneration of any given hydrogen ion concentration is practically the same for all stages of regeneration (p. 488; figs. 8, 11, 12, 14, 16, and 18).

3. Unfavorable hydrogen ion concentrations inhibit regeneration directly by affecting the regenerating tissue, and indirectly by affecting the tadpole as a whole. The direct effect is relatively independent of the size of the tadpole, the indirect effect is greater in smaller tadpoles (p. 498).

4. In water of low oxygen content both the rate of regeneration and the total amount regenerated are dependent upon the amount of oxygen present (table 7; figs. 21, 22, and 23).

5. Both the rate of regeneration and the total amount regenerated decrease with a decrease in temperature (table 8; fig. 24).

6. The phenomena of differentiation in regenerating tissues, as indicated by the appearance of pigmentation, are not retarded to the same extent by unfavorable hydrogen ion concentrations, insufficient oxygen, or low temperatures as are the phenomena of growth or elongation. This, it is suggested, may explain the decrease in the total amount ultimately regenerated due to the unfavorable environmental factors studied (p. 504).

7. Carbon-dioxide production is increased by bases and decreased by acids. A high rate of carbon-dioxide production, when induced by á basic medium, is not correlated with a high rate of regeneration (table 3; figs. 7, 8, 9, 10, 11, and 12).

8. Both the relative decrease of carbon-dioxide production in acids and the relative increase of carbon-dioxide production in bases increase as the size of the tadpole decreases (p. 497).

9. Although the extent to which any environmental factor may affect a given life process may vary with size, age, etc. (p. 497), and although different processes in the same individual may be affected to a different extent by the same environmental factor (p. 504), it is suggested that unsuitable hydrogen ion concentrations, insufficient oxygen, low temperatures, and toxic substances affect development, regeneration, oxygen metabolism, and duration of life in the same way and according to the same laws.

This work was done in the Zoological Laboratory of the University of Illinois, under the direction of Dr. V. E. Shelford, to whom the writer is indebted for many courtesies and valuable

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SUBJECT AND AUTHOR INDEX